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Determination of N-methylcarbamate pesticides in environmental water samples using automated on-line trace enrichment with exchangeable cartridges and high-performance liquid chromatography

M. Hiemstra, A. de Kok*

Food Inspection Service, Department of Pesticide Analysis, Burgpoelwaard 6, 1824 DW Alkmaar, Netherlands

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Abstract

A fully automated high-performance liquid chromatographic (HPLC) method was developed for the determination of N-methylcarbamate pesticides and their polar metabolites in various types of water. Two different, commercially available, automated trace enrichment devices (OSP-2 and Prospekt) were investigated as to their performance. Low-carbon C_{18}-bonded silica (C_{18}/OH, 40 μm particle size) was used, as a selective sorbent for solid-phase extraction and preconcentration of N-methylcarbamate pesticides from environmental water samples. PLRP-S (15–25 μm), a styrene–divinylbenzene polymeric phase, was also evaluated as an alternative to extract N-methylcarbamates.

After preconcentrating the N-methylcarbamates on an exchangeable cartridge, the analytes were eluted with the mobile phase gradient and transferred to the reversed-phase HPLC system. Detection was performed via post-column hydrolysis on a solid-phase catalyst (anion exchanger), derivatization of the methylamine formed with *o*-phthalaldehyde reagent, and fluorescence detection of the isoindole derivative.

Both trace enrichment systems showed good quantitative results for the determination of N-methylcarbamates and their metabolites at the 0.1 μg/l level. The repeatability was excellent with relative standard deviations in the range 2–10%. The method detection limits for surface water were between 30 and 50 ng/l. Sample throughput is about 30 samples per 24 h, unattended. The method proved to be suitable for monitoring N-methylcarbamate pesticides in environmental water samples.

1. Introduction

The ubiquitous presence of pesticides and organic pollutants in environmental water samples has arisen from extensive agricultural application and industrial emission in surface waterways. Therefore, monitoring the trace levels of pesticides and organic pollutants is crucial for human health protection and environmental control. The EEC has set a maximum admissible concentration of 0.1 μg/l for individual pesticides and their related compounds in drinking water [1]. As a consequence, regulatory agencies and drinking water laboratories have shown increased interest in monitoring pesticides in environmental water samples. Multiresidue methods have been established for screening these compounds in drinking, surface and waste

* Corresponding author.

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water, using both gas chromatography (GC) and liquid chromatography (LC).

The popularity of LC has grown rapidly in recent years owing to its suitability for the determination of relatively polar pesticides and it is widely accepted as a rapid and efficient technique. Screening of polar pesticides in water samples is mainly performed by employing HPLC in combination with diode array detection or UV detection [2–5]. In water analysis, emphasis is generally laid on the determination of herbicides (triazines, phenoxycarboxylic acids, phenylureas). This is reflected in the development of various methods for these typical pesticide/matrix combinations. Only a small proportion of analytical work on monitoring residues in water samples concerns the N-methylcarbamate pesticides. McGarvey [6] reviewed the literature concerning all aspects of determination of N-methylcarbamate residues in water, plants, and air by HPLC, including extraction, clean-up, chromatographic separation and detection.

To enhance sensitivity in water analysis, a preconcentration step, prior to LC analysis, is required. Compared with laborious and solvent-consuming liquid–liquid extraction methods, solid-phase extraction has gained in popularity. The most popular sorbents for isolating pesticides from water samples are octadecyl-bonded silica (C_{18}) [4], styrene–divinylbenzene copolymers (PLRP-S, PRP-1) [2,3,5,7] and graphitized carbon [8].

Preconcentration of the N-methylcarbamates from water samples [6] is mainly performed using conventional liquid–liquid extraction or solid-phase extraction. Very recently [9], we developed a multiresidue HPLC method for the determination of 20 N-methylcarbamate pesticides and 12 of their polar metabolites in surface water via solid-phase extraction at low ng/l levels. A new low-carbon C_{18}-bonded silica (C_{18}/OH), specially designed for polar metabolites of pharmaceuticals, was studied. Although off-line solid-phase extraction appeared to be satisfactory, some drawbacks still remain: possible losses of the analyte in the evaporation/redissolution step, loss of sensitivity due to dilution of the eluate and cost effectiveness.

More recently, both direct large-volume aqueous injections [10] and on-line trace enrichment [11–15] of N-methylcarbamates have been demonstrated by several research groups. She *et al.* [11] reported a LC method for the determination of carbaryl by using on-line preconcentration. Another LC method, reported by Chaput [12], determined aldicarb and its metabolites in water, using on-line trace enrichment. Marvin and coworkers applied their on-line preconcentration method to propoxur, carbofuran, carbaryl [13,14] and to aldicarb and its metabolites [15]. In spite of the improvements, several shortcomings still exist. Due to differences in polarity of the N-methylcarbamates and their metabolites, recovery and sensitivity were not always satisfactory for each single compound. The most studied water types were ground, well, pond and drinking waters. Surface water samples have infrequently been investigated. Most studies were done for only a limited number of N-methylcarbamates.

Chiron *et al.* [16] reported a promising trace enrichment method based on the on-line coupling of C_{18}-bonded silica Empore membrane extraction disks (4.6 mm I.D.; particle size 8 μm), stacked in a fixed membrane-disk holder and LC in combination with post-column reaction detection. Samples of 10 ml of surface water could be concentrated for the determination of 15 selected N-methylcarbamates in river water. The determination limits that could be obtained for the carbamates were 0.01 μg/l. The method was less suitable for routine monitoring water samples, because the membrane extraction disks had to be manually replaced after two runs.

With the introduction of commercially available systems for automated on-line trace enrichment (OSP-2 from Merck, Darmstadt, Germany and Prospekt from Spark Holland, Emmen, Netherlands), shortcomings of off-line solid-phase extraction can be avoided and sample volumes can be further reduced. On-line trace enrichment techniques use a precolumn containing an appropriate sorbent, which selectively retains the compounds of interest. A water sample is enriched on an exchangeable cartridge

and subsequently the preconcentrated analytes are desorbed and transferred to the analytical column using the mobile phase gradient.

The aim of this study was to automate the solid-phase extraction of N-methylcarbamate pesticides from water samples to make the method more suitable for monitoring purposes. Special attention has been paid to include the important polar sulphoxide and sulphone metabolites of butocarboxim, aldicarb, ethiofencarb, thiofanox and methiocarb. This paper describes the conversion of the off-line method into an automated on-line trace enrichment method by means of commercially available apparatus (OSP-2 and Prospekt).

2. Experimental

2.1. Chemicals

HPLC-grade acetonitrile and methanol were purchased from Rathburn (Walkerburn, UK) and water was purified using an ElgaStat UHQ water-purification system (Elga, High Wycombe, UK). Glacial acetic acid, sodium acetate, sodium thiosulphate, *o*-phthalaldehyde (OPA), 2-mercaptoethanol and disodium tetraborate were obtained from Merck.

OPA reagent was prepared by dissolving 1.0 g of disodium tetraborate in approximately 200 ml of purified water in a 250-ml volumetric flask. A solution of 50 mg of OPA in *ca.* 2 ml of acetonitrile and 0.1 ml of 2-mercaptoethanol were added. The solution was diluted to 250 ml with water.

All carbamate pesticide and metabolite standards were supplied by Promochem (Wesel, Germany), the Environmental Protection Agency Repository (Research Triangle Park, NC, USA) or the pesticide manufacturers.

2.2. Apparatus

Trace enrichment was executed by means of an OSP-2 on-line sample preparator from Merck, combined with a L-6200 intelligent pump and an AS-4000 autosampler, installed with a 5-ml loop or a Prospekt on-line sample preparation system from Spark Holland, consisting of the Prospekt apparatus, a solvent delivery unit (SDU) and a Marathon autosampler, equipped with a peristaltic pump and a 3-ml loop.

The HPLC analyses were performed with a Hewlett-Packard (Waldbronn, Germany) HP 1050 pumping system, equipped with a Rheodyne (Berkeley, CA, USA) Model 7125 six-port injection valve and 100-μl injection loop, an analytical column oven (35°C), a Kratos (Ramsey, NJ, USA) PCRS 520 post-column reaction system, equipped with a low-dead-volume T-piece, a Hewlett-Packard HP 1050 isocratic pump for OPA reagent delivery and a Hewlett-Packard HP 1046A double monochromator fluorescence detector. Data acquisition and processing were performed on a HP Vectra 486 computer using Hewlett-Packard ChemStation software.

Analytical separations were performed on a Merck LiChroCART 250 × 4.0 mm I.D. cartridge column packed with Supersphere RP-8 (4 μm) from Merck. A Merck LiChroCART 10 × 4.0 mm I.D. guard column packed with Supersphere RP-8 (4 μm) was installed in front of the analytical column.

The post-column reactor consisted of a 50 × 4.0 mm I.D. stainless-steel column packed with Aminex A-27 (15 μm) from Bio-Rad Labs. (Richmond, CA, USA) and was kept at a reaction temperature of 120–140°C. After the catalytic hydrolysis reactor, OPA reagent was added to the eluent at a flow-rate of 0.1 ml/min via a low-dead-volume T-piece. The reaction of methylamine with OPA reagent, to form the isoindole derivative, took place in a 20 cm × 0.12 mm I.D. PTFE connection capillary to the fluorescence detector. The excitation and emission wavelengths were set at 340 and 445 nm, respectively.

2.3. Procedures

Stock solutions (1.0 mg/ml) were prepared by weighing *ca.* 10–15 mg of the standard pesticide and dissolving this amount in an equivalent volume (in ml) of dichloromethane. The stock

solutions were stored at − 18°C in a freezer. A standard mixture (1 μg/ml) was prepared by allowing 100 μl stock solution of each carbamate to evaporate in the air, followed by dissolution in 100 ml of acetonitrile. The standard mixture was stored in the dark, in a refrigerator at 4°C. Preparation of the internal standard solution (trimethacarb) was performed in the same way as the preparation of the standard mixture. Every day a fresh working standard solution (5 ng/ml) for direct standard injections was prepared by diluting 50 μl standard mixture to a volume of 10 ml. For fortification studies, a volume of 100 μl of standard mixture and 100 μl of internal standard solution were diluted with 1 l of blank surface or drinking water. All the aqueous standard solutions and water samples contained 0.1% glacial acetic acid to prevent hydrolysis of the carbamates. When analyzing hypochlorite-containing drinking water, hypochlorite was reduced by adding 0.5 g/l of sodium thiosulphate to avoid oxidation of the carbamates.

Chromatographic runs were performed using a ternary gradient profile. The mobile phase solvents were (A) acetonitrile–water (20:80), (B) methanol–water (20:80) and (C) acetonitrile–water (60:40). To prevent tailing of the methylamine formed during hydrolysis in the catalytic reactor, 2.5 m*M* of sodium acetate was added to each of the mobile phase solvents. Prior to use, all mobile phase solvents were filtered through a 0.45-μm filter applying a vacuum. The following linear gradient program, at a flow-rate of 0.75 ml/min, was run: 75% A and 25% B was kept for 5 min, then linearly to 100% C in 20 min and held for 5 min. Before the next injection, the initial mobile phase composition was held for 15 min.

Three types of precolumns were evaluated in this study: (1) precolumns (10 × 4.0 mm I.D.) for the OSP-2, manually dry-packed in our laboratory with 40-μm C_{18}/OH Bondesil (Varian/Analytichem, Harbour City, CA, USA), containing 55 mg of packing material; (2) precolumns (10 × 3.0 mm I.D.) for the Prospekt, dry-packed with 40-μm C_{18}/OH Bondesil by Spark Holland, containing 30 mg of packing material; (3) commercially available precolumns (10 × 3.0 mm I.D.) for the Prospekt, slurry-packed with 15–25 μm PLRP-S (Polymer Labs.) by Spark Holland.

All precolumns, packed with C_{18}/OH, were desorbed applying a forward-flush. Precolumns for the Prospekt, packed with PLRP-S, were desorbed applying a back-flush.

All cartridges were activated with 1 ml methanol followed by 1 ml of purified water at a flow-rate of 1 ml/min using either the L-6200 pump or the SDU. During the activation of the precolumn, the injection loop of the autosampler was loaded with the water sample. With the injection valve of the autosampler (Marathon or AS-4000) in the inject position, an appropriate volume (3–5 ml) of water sample was passed through the precolumn at a flow-rate of 1 ml/min. An additional volume of 0.5 ml of distilled water was allowed to pass through the precolumn in order to remove some matrix interferences. The whole trace enrichment procedure was executed within the equilibration time of the analytical column. The preconcentrated carbamates were then desorbed by switching the solid-phase extraction cartridge on-line with the HPLC system and starting the mobile phase gradient profile. Subsequently, all connection capillaries of the trace enrichment system were flushed with methanol. For further technical details of the valve switching schemes of both the OSP-2 and Prospekt, see Fig. 1.

3. Results and discussion

3.1. Chromatography and automation

In Table 1, the retention times of twenty N-methylcarbamates and twelve metabolites in the reversed-phase HPLC system Supersphere RP-8 (4 μm) with an acetonitrile–methanol–water gradient are presented. A detailed discussion of the optimized reversed-phase HPLC separation, using the linear gradient and the post-column derivatization system, using the catalytic solid-phase column, is given in ref. 9.

A review of the literature [6] shows, that UV

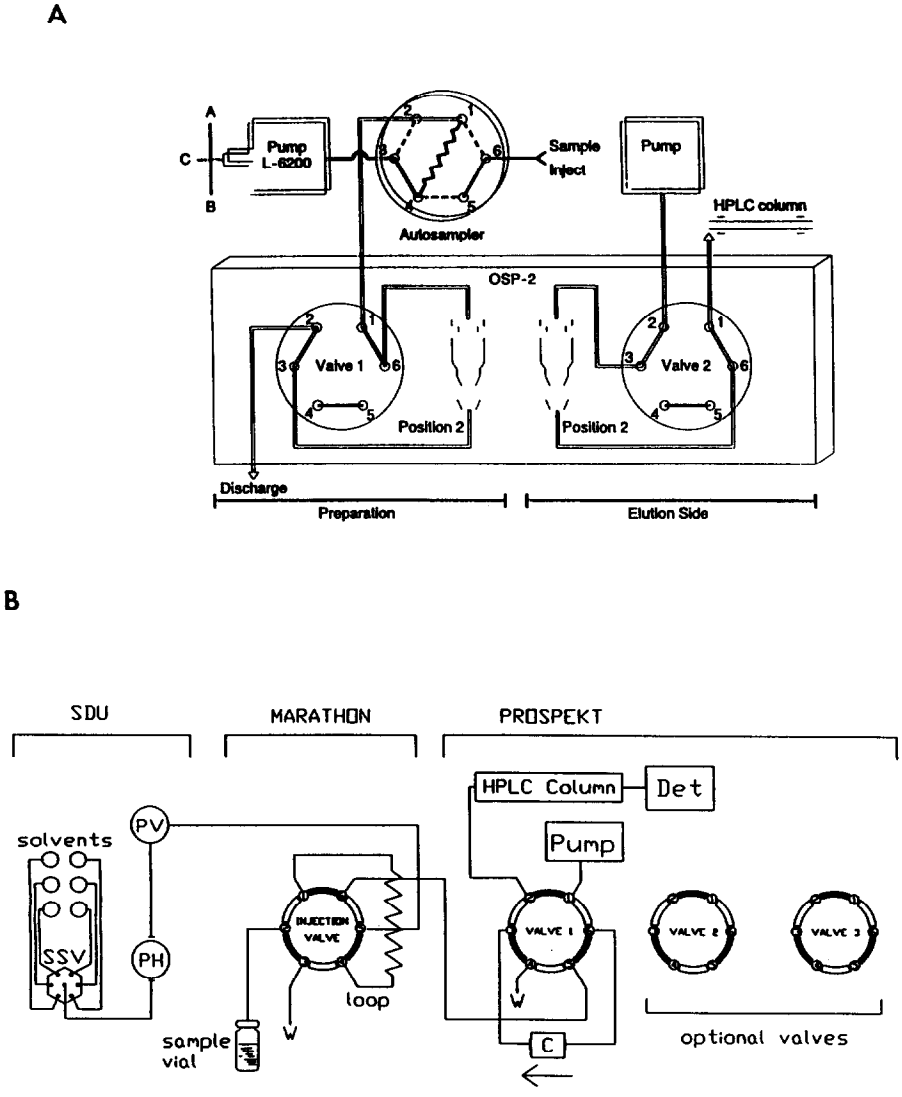

Fig. 1. Details of the valve switching systems of both the (A) OSP-2 system and (B) Prospekt system. SSV = Solenoid valve; PH = purge pump; PV = pulse damper; C = cartridge; DET = detection system; W = waste.

absorbance is the most commonly applied detection method for the determination of N-methylcarbamates in water. However, matrix interferences limit its usefulness due to lack of selectivity. Besides, sensitivity has not always been sufficient. In order to overcome these limitations, we have employed selective post-column derivatization of the N-methylcarbamates followed by sensitive fluorescence detection. A uniform response for all carbamates and metabolites is an additional advantage. Although this technique is very sensitive and selective, trace enrichment is still required, in order to meet with EEC limit concentrations. Preconcentration procedures prior to HPLC are usually performed manually applying liquid–liquid extractions or solid-phase extractions. The present availability of automated on-line trace enrich-

Table 1
Retention times of twenty N-methylcarbamate pesticides and twelve metabolites in the reversed-phase HPLC system Supersphere RP-8 with an acetonitrile–ethanol–water gradient

Carbamate/metabolite	Retention time (min)
Butocarboxim sulphoxide	5.53
Aldicarb sulphoxide	5.95
Butocarboxim sulphone	8.11
Aldicarb sulphone	8.80
Oxamyl	9.44
Methomyl	11.70
Ethiofencarb sulphoxide	12.97
Thiofanox sulphoxide	13.35
Ethiofencarb sulphone	14.99
3-Hydroxycarbofuran	15.00
Methiocarb sulphoxide	15.11
Tranid	15.15
Dioxacarb	16.45
Thiofanox sulphone	16.65
Methiocarb sulphone	18.76
Butocarboxim	19.30
3-Ketocarbofuran	20.05
Aldicarb	20.21
Cloethocarb	22.95
Propoxur	23.09
Carbofuran	23.36
Bendiocarb	23.36
Carbaryl	24.53
Thiofanox	25.00
Ethiofencarb	25.06
Isoprocarb	26.09
Trimethacarb	26.12
Carbanolate	26.75
Methiocarb	28.48
Fenobucarb	28.69
Promecarb	29.60
Bufencarb	33.52

ment systems (OSP-2, Prospekt) on the market makes the method more suitable for monitoring purposes. Both systems can perform trace enrichment of compounds from water utilizing exchangeable cartridges, unattended. Before the injection of a water sample the systems automatically insert a new solid-phase extraction cartridge, select solvents and then start the sample introduction and preparation. The systems complete the sample preparation within the analysis time of the previous sample, saving time normally used for sample preparation.

3.2. Stability studies

It has been reported [17] that carbamate and carbamoyl oxime pesticides can be degraded by acid- and base-catalyzed hydrolysis in water. Chlorinated water shortened the half-lives of all the carbamates. The effect of chlorination on half life was greater at pH 8 than pH 7. In our laboratory, carbamate stability studies were performed on untreated surface water and drinking water. Within one week, losses for all N-methylcarbamates and metabolites were observed. Especially in chlorinated water samples, the degradation of butocarboxim, aldicarb, ethiofencarb, thiofanox and methiocarb and their corresponding metabolites begins within hours. These results indicate that water contaminated with carbamates/metabolites and treated by chlorination will contain lower concentrations of these pesticides. Therefore, the analysis of N-methylcarbamates in surface water and chlorinated drinking water, requires an addition of acid/preservative directly at the sampling site, in order to prevent hydrolysis/oxidation of the carbamates. Addition of 0.1% glacial acetic acid and 0.5 g/l sodium thiosulphate to the water samples did not affect the performance of the system.

3.3. Solid-phase materials

In our previous study [9], an off-line solid-phase extraction procedure was developed, based on preconcentration of the N-methylcarbamates on C_{18}/OH cartridges. Most research groups use C_{18}-bonded silica cartridges to enrich the compounds of interest. Chaput [12] and Lesage [18] have demonstrated that C_8 material is preferred over C_{18} material for preconcentrating polar carbamates from water samples, due to its good solute retention capacity. We have shown that C_{18}/OH material has even a much better selectivity for the most polar carbamates and metabolites. This can possibly be ascribed to the number and/or accessibility of the free silanol groups on the surface of the sorbent. In on-line trace enrichment, precolumns packed with 3–10 μm particles are often used. Blockage

of the these precolumns can often occur owing to restrictions imposed by the high column back pressure at high sampling flow-rates or prolonged time of sampling. With the use of larger particles (40 μm) blockage of the precolumns is less likely to occur.

Apart from C_{18}/OH, PLRP-S, a styrene–divinylbenzene copolymer, was also studied, because PLRP-S is widely employed for the on-line trace enrichment of pesticides from water [2,7].

3.4. Recovery studies

For recovery studies, we selected those carbamates/metabolites which were most likely to occur in surface water samples and drinking water samples, because they are produced in significant quantities at industrial sites and applied intensively in agriculture.

Using 10 × 4.0 mm I.D. C_{18}/OH precolumns for the OSP-2, breakthrough volumes of a surface water sample spiked with N-methylcarbamates at 0.1 μg/l were assessed. It appeared that a sample volume of 6 ml could be preconcentrated before breakthrough of the polar carbamates or metabolites occurred. This was consistent with breakthrough volumes obtained with our off-line method. For recovery studies, a 5-ml water sample fortified with 18 selected N-methylcarbamates at the 0.1 μg/l level, was passed through the precolumns. The recoveries of the N-methylcarbamates from drinking and surface water samples were measured by comparing the peak heights of a 100-μl loop injection with the peak heights after enrichment and desorption from the precolumns. The results obtained are given in Table 2. The recoveries for the C_{18}/OH precolumn, using the OSP-2 apparatus, were in the range of 82.3–111.0% (R.S.D. 2.6–9.4%) and 79.9–102.9 (R.S.D. 2.0–8.1%) for drinking water and surface water,

Table 2
Average percent recoveries and relative standard deviations (%, in parentheses; $n = 7$) of N-methylcarbamates and metabolites from drinking and surface water, fortified at 0.1 μg/l level, after on-line trace enrichment

Carbamate/metabolite	Prospekt On-line trace enrichment of 3 ml of drinking water			OSP-2 On-line trace enrichment of 5 ml of	
	PLRP-S 10 × 2.0 mm	PLRP-S 10 × 3.0 mm	C_{18}/OH 10 × 3.0 mm	Drinking water C_{18}/OH 10 × 4.0 mm	Surface water C_{18}/OH 10 × 4.0 mm
Butocarboxim sulphoxide	73.4 (8.6)	96.1 (7.5)	95.0 (7.1)	97.9 (8.6)	98.0 (6.3)
Aldicarb sulphoxide	75.7 (8.8)	95.5 (7.7)	96.4 (7.5)	97.3 (8.6)	99.3 (6.0)
Butocarboxim sulphone	93.2 (8.2)	91.3 (9.2)	90.9 (7.3)	82.3 (8.2)	79.9 (5.7)
Aldicarb sulphone	92.3 (7.3)	93.2 (5.4)	87.3 (6.4)	92.3 (7.3)	84.1 (5.6)
Oxamyl	101.0 (7.5)	91.5 (8.0)	97.0 (7.5)	111.0 (7.5)	97.4 (6.8)
Methomyl	91.9 (6.9)	99.8 (6.1)	91.6 (5.4)	96.3 (6.9)	85.6 (4.5)
Ethiofencarb sulphoxide	94.9 (7.9)	94.7 (5.9)	102.0 (6.7)	98.9 (7.9)	99.9 (3.7)
Thiofanox sulphoxide	n.d.	n.d.	n.d.	89.6 (7.5)	97.6 (7.6)
Methiocarb sulphoxide	91.7 (3.6)	108.4 (4.3)	102.0 (5.7)	101.9 (3.6)	99.1 (3.3)
Thiofanox sulphone	n.d.	n.d.	n.d.	103.7 (3.6)	97.1 (3.2)
Methiocarb sulphone	98.5 (4.7)	104.5 (5.9)	97.3 (6.3)	107.3 (3.7)	96.4 (4.9)
Butocarboxim	96.9 (6.4)	96.4 (5.8)	80.1 (9.6)	110.1 (9.4)	101.6 (8.1)
Aldicarb	99.4 (5.1)	97.4 (5.2)	98.3 (4.8)	101.7 (5.1)	95.6 (2.0)
Propoxur	96.4 (2.6)	101.5 (4.0)	103.1 (4.2)	97.1 (2.6)	100.6 (4.0)
Carbofuran	98.6 (3.1)	104.2 (5.3)	101.9 (5.0)	98.9 (3.1)	98.9 (5.3)
Carbaryl	97.3 (2.9)	96.6 (4.2)	102.1 (3.6)	98.6 (2.9)	98.6 (5.2)
Ethiofencarb	54.0 (9.4)	60.0 (7.9)	100.1 (2.5)	99.7 (3.4)	102.9 (2.8)
Methiocarb	86.9 (5.0)	82.9 (4.8)	89.1 (3.8)	89.3 (5.0)	92.4 (5.1)

n.d. = Not determined.

respectively. Fig. 2 shows a typical chromatogram of a surface water sample fortified with the standard mixture of 18 N-methylcarbamates at 0.1 μg/l after extracting 5 ml of sample, using the OSP-2 apparatus. In spite of the use of a precolumn packed with 40 μm particles, no extra band broadening was observed for the early-eluting polar carbamates. During desorption of the carbamates, the polar carbamates were reconcentrated at the head of the analytical column and began to elute only when the proportion of organic solvent in the gradient mobile phase increased, thereby preventing any loss of separation efficiency.

From the breakthrough volumes assessed for our off-line procedure and for the 10 × 4.0 mm I.D. precolumns used with the OSP-2, we could derive that it should be possible to preconcentrate a sample volume of 3 to 4 ml on a 10 × 3.0 mm I.D. precolumn, packed with C_{18}/OH, using the Prospekt. The retention capacity of PLRP-S was compared with C_{18}/OH, both packed in 10 × 3.0 mm I.D. precolumns. For recovery studies, a 3-ml drinking water sample, fortified with the standard mixture of 16 N-methylcarbamates at a level of 0.1 μg/l, was loaded on both precolumns. The results obtained are given in Table 2. Recoveries for the C_{18}/OH and PLRP-S precolumns, using the Prospekt apparatus, ranged from 80.1–103.1% (R.S.D. 2.5–9.6%) and 60.0–108.4% (R.S.D. 4.0–9.2%), respectively. Surprisingly, ethiofencarb was not com-

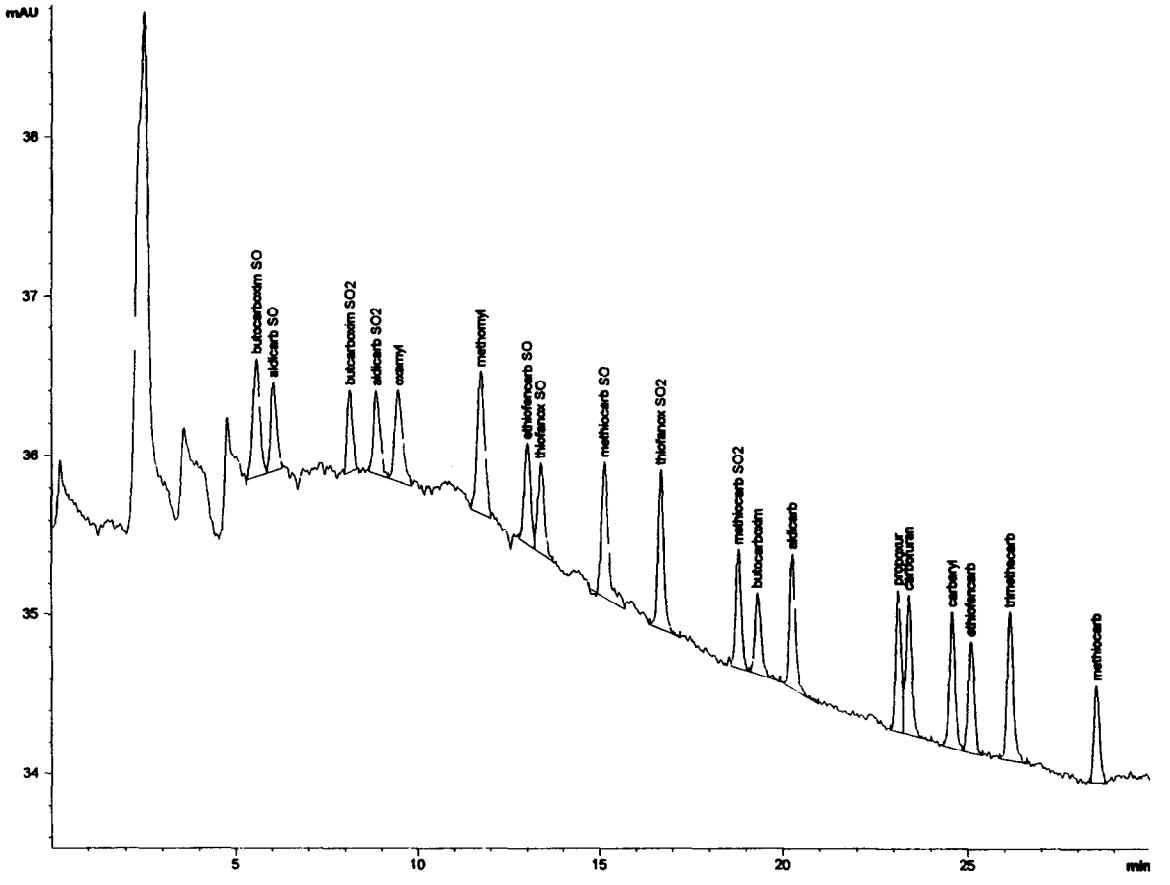

Fig. 2. HPLC chromatogram of a 5-ml surface water sample, fortified with 18 N-methylcarbamates and metabolites at the 0.1 μg/l level, after on-line trace enrichment on a 10 × 4.0 mm I.D. C_{18}/OH precolumn, using the OSP-2 system. Trimethacarb was used as an internal standard.

pletely recovered on the PLRP-S sorbent. No explanation can be given for this deviating behaviour.

A closer look at Table 2 shows that the recoveries and the repeatability obtained with the C_{18}/OH precolumns, using both automated systems, are in good agreement with one another. In comparison with the results of our previously developed off-line method [9], the relative standard deviations of the on-line method are somewhat higher, but still well below 10%. The major advantages of the automated on-line method with exchangeable solid-phase extraction cartridges are the reduced sample size required (typically 3–5 ml), a high sample throughput (30 samples per 24 h) and the absence of cross-contamination from one sample to the next one. For all N-methylcarbamates and their metabolites, the linear dynamic range of the trace enrichment procedure, using the OSP-2, was checked by using fortified surface water (0.05–1 μg/l). The average coefficient of correlation was 0.995. Method detection limits (based on a signal-to-noise ratio 3:1) were in the range 0.03–0.05 μg/l for both automated systems tested. In recent measurements with newer types of fluorescence detectors, these limits could even be lowered by a factor of three.

3.5. Comparison of C_{18}/OH and PLRP-S

A comparison of the retention capacity of the 40-μm C_{18}/OH sorbent and the 15–25-μm PLRP-S sorbent revealed no significant differences in the retention capacity for the polar carbamates. To maintain separation efficiency, the sorbent of the precolumn should have a hydrophobicity similar to that of the analytical column. In comparison with the chromatogram obtained with a direct loop injection, PLRP-S precolumns, using forward-flush, caused substantial band broadening for the later-eluting carbamates, resulting in loss of separation efficiency. This indicates that the non-polar carbamates are retained more by the PLRP-S sorbent than by the stationary phase of the analytical column. It is therefore clear that the elution efficiency of the C_{18}/OH sorbent is better than the PLRP-S sorbent. When applying a back-flush, band broadening of the non-polar carbamates does not occur. However, a major drawback of this procedure is that matrix interferences are also eluted onto the guard column, causing build up of strongly retained compounds on the guard column during analysis. Fig. 3 depicts the chromatograms of a standard mixture of 16 N-methylcarbamates extracted from 3 ml of drinking water fortified with the carbamates at the 0.1 μg/l level on both phases, using the Prospekt apparatus. Desorption of the PLRP-S precolumn was performed by applying a back-flush.

We have also studied the use of precolumns, packed with PLRP-S, with a smaller diameter (2.0 mm), in order to increase the elution efficiency and therefore minimize the loss of separation efficiency. In contrast to the results with 10×3 mm I.D. PLRP-S precolumns, with $10 \times$ 2.0 mm I.D. PLRP-S precolumns the resolution between the non-polar carbamates was completely maintained, using forward-flush desorption. The recovery and precision results obtained are also given in Table 2. Despite the incomplete recoveries of the polar metabolites butocarboxim sulphoxide and aldicarb sulphoxide due to breakthrough, the repeatability was still good. Hence, precolumns, packed with PLRP-S, proved to efficiently extract N-methylcarbamates from water samples and therefore can serve as a good alternative to C_{18}/OH precolumns. However, the cost of the larger C_{18}/OH particles (40 μm) is somewhat lower than for the polymeric PLRP-S particles (15–25 μm).

3.6. Applications

During a monitoring period of six months, river Rhine water samples were analyzed for N-methylcarbamates and their metabolites, using the on-line trace enrichment method. Fig. 4 depicts the chromatograms obtained using the OSP-2 system. No detectable residues of N-methylcarbamate pesticides or their metabolites were observed. However, during the last months, unknown peaks appeared in the chromatograms. No effort was made yet to trace their identity. Elucidation of the identity of these

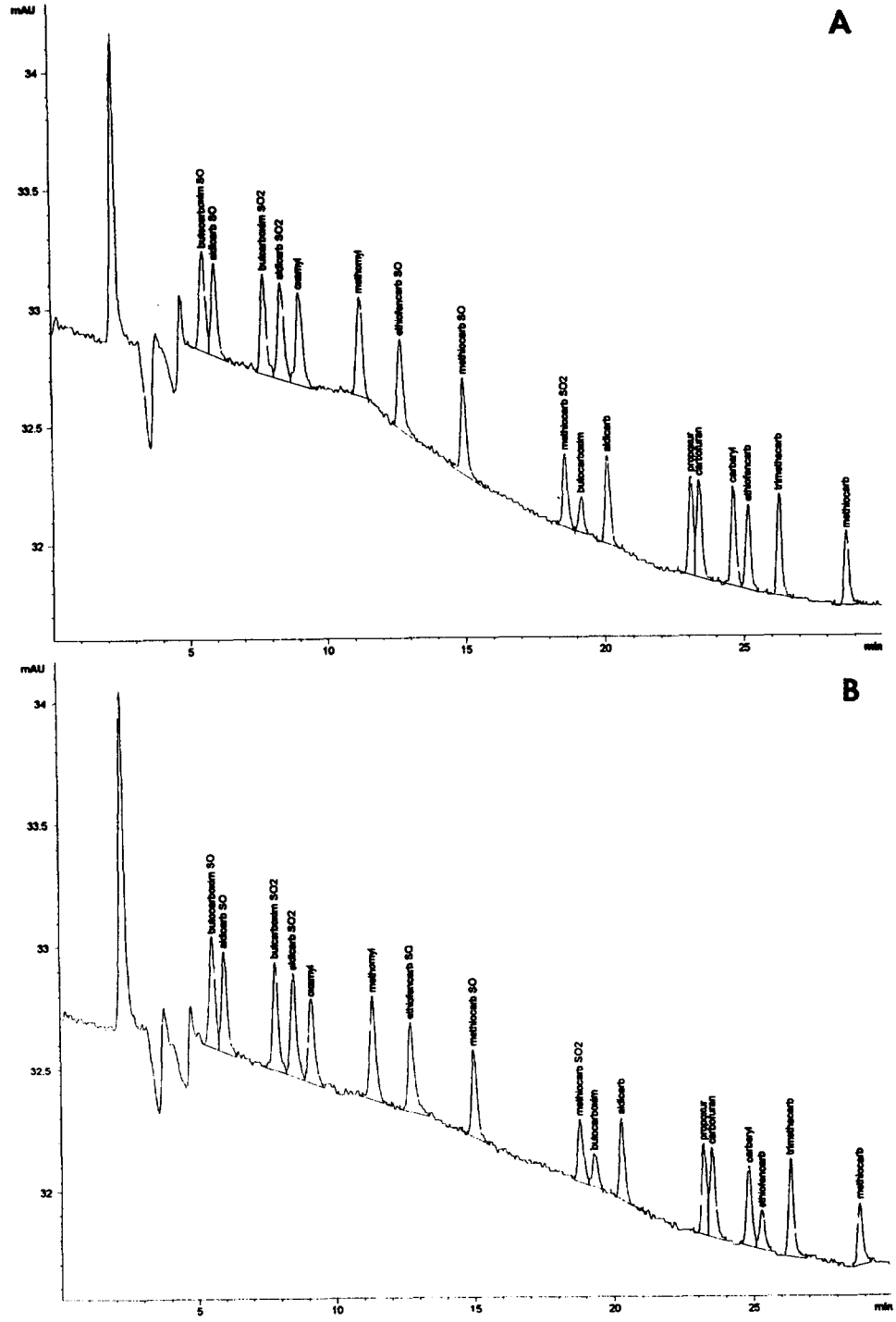

Fig. 3. HPLC chromatograms of a 3-ml drinking water sample, fortified with 16 N-methylcarbamates and metabolites at the 0.1 μg/l level, after on-line trace enrichment on (A) a 10 × 3.0 mm I.D. C_{18}/OH precolumn and (B) a 10 × 3.0 mm I.D. PLRP-S precolumn, using the Prospekt system. Desorption of the PLRP-S precolumn was performed by applying a back-flush. Trimethacarb was used as an internal standard.

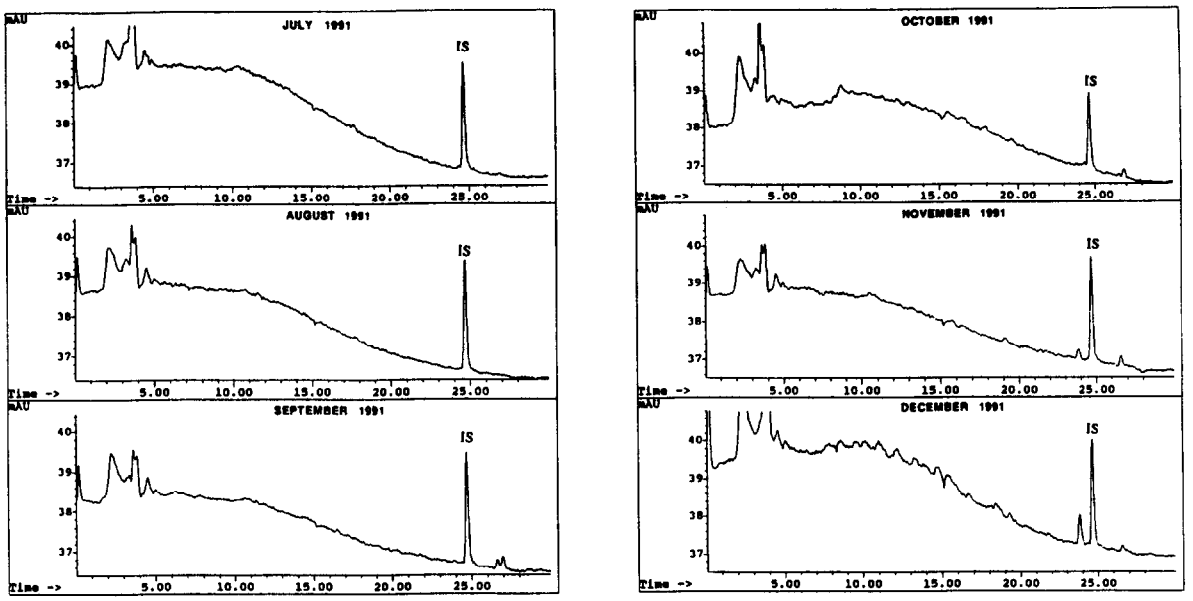

Fig. 4. HPLC chromatograms resulting from the analysis of river Rhine water samples, during a monitoring period of 6 months. The samples were analyzed using the OSP-2 system. Trimethacarb was used as an internal standard (IS). None of the peaks correspond to N-methylcarbamate standards available to us. Time in min.

unknown compounds would require the use of LC in combination with mass spectrometry [16] or diode array detection [4].

4. Conclusions

A fully automated solid-phase extraction–HPLC method has been developed for the determination of N-methylcarbamates and their metabolites in environmental water samples at low ng/l levels. Automated on-line trace enrichment apparatus (OSP-2 and Prospekt) can be effectively applied for extracting and preconcentrating trace levels of N-methylcarbamates from various types of water. Recoveries are high (80–111%), the repeatability is quite good (R.S.D. 2–10%) and a high sample throughput can be achieved (30 samples per 24 h). Method detection limits for all tested N-methylcarbamates were in the range of 0.03–0.05 μg/l.

As was already shown in the off-line method, C_{18}/OH (40 μm) offers distinct advantages over C_{18}-, C_8-bonded silica as to retention efficiency. PLRP-S appears to be a good alternative for the preconcentration of N-methylcarbamates from water samples. The C_{18}/OH phase has a superior selectivity for the more polar carbamates/metabolites and could have a much broader applicability in polar pesticide analysis in the future.

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