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Low ng/l-level determination of twenty N-methylcarbamate pesticides and twelve of their polar metabolites in surface water via off-line solid-phase extraction and high-performance liquid chromatography with post-column reaction and fluorescence detection

A. de Kok and M. Hiemstra

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

U. A. Th. Brinkman

Department of Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam (Netherlands)

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ABSTRACT

Reversed-phase high-performance liquid chromatography (HPLC) was used for the determination of twenty parent N-methylcarbamate pesticides and twelve major metabolites in surface water. A 50-ml water sample was passed through a disposable solid-phase extraction cartridge, packed with 500 mg of low-carbon C_{18}-bonded silica (C_{18}/OH, 40 μm particle size), which selectively retained polar compounds. The preconcentrated analytes were eluted with acetonitrile, reconstituted in 1 ml of water and 100 μl were injected into the HPLC system. The carbamates were separated via a water–methanol–acetonitrile gradient. Detection was performed via postcolumn hydrolysis on a solid-phase (anion-exchange) catalyst, derivatization of the methylamine formed with *o*-phthalaldehyde–2-mercaptoethanol and fluorescence detection of the isoindole derivative. The detection limits for surface water were between 20 and 30 ng/l. Recoveries were determined for thirteen carbamates and ten metabolites at the 0.1 and 1 μg/l level and generally ranged from 76 to 106% with relative standard deviations (R.S.D.s) of 0.5–8.5%. Only the sulphoxide metabolite of ethiofencarb and thiofanox had lower recoveries and correspondingly higher R.S.D.s.

INTRODUCTION

The worldwide increase in the use of pesticides during the last two decades has led to the presence of residues of these pesticides not only on the crops to which they are applied but also, owing to leaching into and run-off from the soil, in ground water and surface waters, respectively. Because of the proven or suspected toxicity of many of these pesticides, their monitoring is needed from both the regulatory and the consumer points of view. Surveillance by governmental agencies of pesticide residues on crops has become routine since the discovery of DDT residues in the early 1960s. However, only in the last 5 years has monitoring of pesticides in ground and surface waters been performed on a regular basis, especially since high residue levels were reported for various ground and well waters. In the USA the National Pesticide Survey project was initiated to conduct a statistically based survey

Correspondence to: Dr. A. de Kok, Food Inspection Service, Department of Pesticide Analysis, Burgpoelwaard 6, 1824 DW Alkmaar, Netherlands.

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of pesticide contamination of drinking-water wells [1].

In crop analysis, emphasis is generally laid on the determination of insecticides (organochlorine and organophosphorus compounds, carbamates) and fungicides (benzimidazoles, phthalimides), whereas in water analysis herbicides (triazines, phenoxycarboxylic acids, phenylureas) are often the target compounds. This is reflected in the availability of analytical methods for these typical matrix–pesticide combinations. Especially in Europe, the analysis of water samples for N-methylcarbamates has only attracted increased attention in recent years.

Since the development of a high-performance liquid chromatographic (HPLC) analysis for N-methylcarbamates by Moye *et al.* [2], based on post-column hydrolysis, followed by derivatization with *o*-phthaladehyde (OPA) reagent and fluorescence detection, this method is now used routinely for food analysis in the FDA laboratories in the USA. The application to food samples has been elaborated by Krause [3,4] in the USA and by de Kok and co-workers [5–7] in the Netherlands. We have further improved the former method by the incorporation of a rapid and effective solid-phase extraction (SPE) clean-up [5] and optimized postcolumn reaction technology [6] based on the efficient hydrolysis of the N-methylcarbamates on a solid phase, *e.g.*, a strong anion-exchange material or magnesium oxide. The method has ultimately been fully automated by combining automated SPE with on-line gradient elution HPLC analysis and data processing [7].

After the occasional detection of some individual carbamates in ground and river water samples, drinking water laboratories in the Netherlands showed increased interest in the possible application of our method for crop samples to surface water samples, after some necessary adaptations. This prompted us to investigate various water sample preparation techniques for the extraction and preconcentration of N-methylcarbamates in water.

HPLC methods for N-methylcarbamates are now generally preferred over gas chromatographic (GC) methods, which will not be considered here. For HPLC, no derivatization is required for the thermolabile N-methylcarbamates and the aqueous samples, analysed either directly or after preconcentration, are very compatible with reversed-phase HPLC. It should be noted that in the early 1980s only residue methods for single, or a limited number of, compounds were developed. Aldicarb and its metabolites [8–12] and, to a lesser extent, carbofuran and its metabolites [12,13], carbaryl and its metabolites [14] and oxamyl and its metabolites [15] have been predominantly studied. These methods have in common that sample preparation was performed either by repetitive extraction of 100–1000-ml samples with dichloromethane [8,10,12,15,16] and dissolution of the evaporated extract in the mobile phase solvent or direct injection [9,11,13,14] of large volumes (0.2–5 ml). UV detection at the absorption maxima (190–205 nm) or secondary absorption maxima (220, 247 or 280 nm) was routinely used. The detection limits ranged between 1 and 10 μg/l.

More recently, both direct large-volume aqueous injections [17–22] and off-line [23–25] and on-line [26–31] SPE techniques have gained in popularity. Using these preconcentration methods, improved detection limits have been obtained. Modern UV detectors or the postcolumn reaction detection principle followed by sensitive fluorescence detection [17–21,25–27] have also led to improved detection limits. Finally, real multi-residue methods for up to twelve N-methylcarbamates have been developed for water analysis [16–21] analogous to the approach for crop sample analysis [3–7].

Notwithstanding these improvements, several shortcomings still exist. The most studied water types are ground, well, pond and drinking waters. Heavily polluted river or surface water samples have hardly been investigated. Method detection limits have decreased to the 0.1–1.0 μg/l range, but owing to the stringent European Community (EC) Directive imposing a tolerance level for all pesticides of 0.1 μg/l, a further increase in sensitivity is still required.

Marvin and co-workers [28,29] reported determination limits between 0.01 and 0.07 μg/l using on-line preconcentration on 3 cm × 4.6 mm I.D. columns. Samples of 100 ml of drinking water could be concentrated for the determination of a group of eleven pesticides, including four N-methylcarbamates (aminocarb, propoxur, carbaryl and carbofuran). The same group [30] developed an advanced, automated SPE method with disposable C_{18} SPE cartridges, using a Waters Millilab Work-

station for the analysis of several tap and surface water samples. Determination limits for propoxur, carbofuran and carbaryl were 0.13, 0.14 and 0.02 μg/l, respectively. In a recent study, Marvin *et al.* [31] applied their on-line preconcentration method to the relatively more polar N-methylcarbamates aldicarb, aldicarb sulphoxide and aldicarb sulphone. Instead of 100 ml, now only 10 ml could be preconcentrated, because greater sample volumes resulted not only in the loss of the polar aldicarb metabolites due to breakthrough, but also gave a poorer separation of these two early eluting analytes. The determination limits that could be obtained for these carbamates were correspondingly higher, namely 7–11 μg/l.

Both McDonald *et al.* [19] and Dong *et al.* [20] have evaluated a promising multipesticide method based on direct aqueous injections with determination limits down to 0.2 μg/l. However, only distilled and well water, respectively, were analysed. The same principle was used by Edgell *et al.* [21] in a collaborative study on the determination of ten carbamates in finished drinking water with reported estimated detection limits of 0.5–4.0 μg/l.

The aim of this study was to develop a considerably extended multi-residue method for virtually all N-methylcarbamates and to include the important sulphoxide and sulphone metabolites of aldicarb, methiocarb, ethiofencarb, butocarboxim and thiofanox. The method should be able to detect the analytes at levels which are a factor 3–5 below the EC tolerance limit of 0.1 μg/l, and it should be applicable to heavily polluted water samples. This has been achieved by combining SPE of water samples with HPLC separation of the carbamates and fluorescence detection after solid-phase-catalysed hydrolysis and derivatization of methylamine with OPA reagent.

EXPERIMENTAL

Chemicals

HPLC-grade acetonitrile and dichloromethane were purchased from Rathburn (Walkerburn, UK) and water was purified with an ElgaStat UHQ water-purification system (Elga, High Wycombe, UK). *o*-Phthalaldehyde (OPA), 2-mercaptoethanol and disodium tetraborate (anhydrous) were obtained from Merck (Darmstadt, Germany).

OPA reagent was prepared by dissolving 2.0 g of disodium tetraborate in *ca.* 500 ml of purified water in a 1-l volumetric flask, adding 250 mg of OPA (dissolved in 1 ml of acetonitrile) and 0.1 ml of 2-mercaptoethanol and diluting to volume with water. The OPA reagent solution and the HPLC mobile phase solvents were degassed under vacuum prior to use.

Carbamate pesticide and metabolite standards were supplied by Promochem (Wesel, Germany) or by the Environmental Protection Agency Repository (Research Triangle Park, NC, USA). Stock solutions (1 mg/ml) were prepared by dissolving *ca.* 10 mg of standard in a suitable volume of dichloromethane. Standard mixtures were prepared by transferring 100 μl of standard solutions, by means of an injection syringe, into a 100-ml volumetric flask and diluting to volume with dichloromethane. The standard solutions are kept in a freezer at −18°C, where they are stable for at least 1 year. For fortification studies, an appropriate volume (10–100 μl) of the standard mixture in dichloromethane was allowed to evaporate in air and the residue was dissolved in blank surface water samples.

Apparatus

Chromatographic separations were effected with a Hewlett-Packard HP 1050 pumping system using a ternary gradient, a variable-volume injector (with a 100-μl loop), a temperature-controlled analytical column compartment (35°C), a Model 7910 (reactor) column oven (Jones Chromatography, Little, CO, USA), a Hewlett-Packard HP 1050 isocratic reagent-delivery pump provided with a special low-pressure pulse damper (Free University, Amsterdam, Netherlands) for postcolumn reagent delivery, a vortex mixing tee-piece (Kratos, Ramsey, NJ, USA) and a Hewlett-Packard HP 1046A double monochromator fluorescence detector.

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.

The postcolumn carbamate hydrolysis column (50 × 4.0 mm I.D.) was packed in our laboratory [6] with Aminex A-27 (15 μm) from Bio-Rad Labs. (Richmond, CA, USA) and kept at a reaction temperature of 120–140°C. The optimum hydrolysis efficiency was obtained when the peak height of

methomyl was as high as that of oxamyl.

After the catalytic hydrolysis reactor, OPA reagent was added to the column effluent at a flow-rate of 0.1 ml/min. The methylamine reacted with the OPA reagent in a 20 cm × 0.12 mm I.D. PTFE capillary connecting the vortex tee-piece with the fluorescence detector inlet. No extra precautions such as cooling the solvent stream prior to fluorescence detection were necessary. The excitation and emission wavelengths were 340 and 445 nm, respectively. The monochromator slit widths were 20 and 15 nm, respectively. It should be noted that with fluorescence detectors from different manufacturers, depending on the flow cell construction and volume and/or the detector outlet capillary length and inner diameter, a back-pressure regulator (*ca.* 10 bar) may be required to prevent boiling of the mobile phase.

Ternary gradient elution runs were as follows. The mobile phase solvents were (A) acetonitrile–water (20:80), (B) methanol–water (20:80) and (C) Acetonitrile–water (60:40) with the following conditions:

Gradient run time (min)	A (%)	B (%)	C (%)
0	65	35	0
5	65	35	0
25	0	0	100
30	0	0	100
32	65	35	0
40	65	35	0

The mobile phase flow-rate was 0.75 ml/min.

Water sample preparation

Water samples were collected from the rivers Rhine and Meuse and, for fortification studies, from the Dutch lake IJsselmeer. In order to prevent degradation of some of the more labile carbamates, the water samples should be conserved [21], *e.g.*, by adding glacial acetic acid to obtain a pH of 3. Samples were stored in a refrigerator (4°C). Filtration of the water samples prior to SPE is not required, provided that the water is allowed to settle.

Liquid–liquid extraction. A 250-ml volume of surface water sample was extracted with three separate 100-ml portions of dichloromethane using a separating funnel and vigorous shaking. The organic phases were combined and concentrated in a rotary evaporator under vacuum to *ca.* 5 ml, then transferred into a centrifuge tube. The extract was evaporated to dryness with a gentle stream of nitrogen and the residue was taken up in 1 ml of distilled water.

Liquid–solid extraction. Bond-Elut SPE cartridges containing 500 mg of adsorbent were obtained from Varian/Analytichem (Harbor City, CA, USA). Three different phases were used: C_8-, C_{18}- and low-carbon C_{18}/OH-bonded silica (particle size 40 μm). SPE experiments were executed by means of a Varian/Analytichem Vac-Elut system, applying a vacuum of 15 kPa. The SPE columns were attached to the vacuum manifold and 75-ml reservoirs were placed on top of the SPE cartridges. The cartridges were conditioned by passing 2 ml of acetonitrile followed by 3 ml of water. Then 50 ml of water sample were passed through the cartridge, which was washed with an extra 3 ml of water. When the water level just reached the top of the column packing, 2 ml of acetonitrile were applied to elute the N-methylcarbamates in a calibrated centrifuge tube, to which 10 μl of an internal standard solution (1 μg/ml landrin in dichloromethane) had already been transferred. The extract was evaporated with nitrogen until *ca.* 200 μl of residual water were left. The extract was made up to 1.0 ml with distilled water.

HPLC analysis

From the final 1-ml SPE extract, 100 μl were injected into the HPLC system. The carbamate concentrations were calculated via the internal standard method. Peak areas of carbamates relative to the peak area of landrin were calculated for both sample extracts and standard mixtures, each containing the same absolute amount of internal standard. Data acquisition and processing were performed via a Hewlett-Packard Chem Station. The fluorescence detector response was linear for injected amounts of 0.1–100 ng for all carbamates studied, with correlation coefficients of between 0.995 and 1.000.

RESULTS AND DISCUSSION

HPLC analysis

Various workers [7,17,20,21] have shown that a multi-pesticide mixture of nine to twelve N-methyl-

carbamates can be separated using a binary gradient of water with methanol or acetonitrile; McDonald *et al.* [19] further improved the critical resolution between the early eluting aldicarb sulphoxide, aldicarb sulphone, oxamyl and methomyl by applying a more complex ternary water–methanol–acetonitrile gradient. Our task however, was, to separate a mixture of 34 compounds including the sulphoxide and sulphone metabolites of aldicarb, butocarboxim, ethiofencarb, methiocarb and thiofanox, and also 3-hydroxy- and 3-ketocarbofuran. These metabolites are relatively polar and emerge in the front part of the chromatogram. With real water samples interferences mostly show up in this region. Therefore, we further optimized the reversed-phase HPLC separation using a linear water–methanol–acetonitrile gradient with an initial isocratic part of 5 min. The relative retention times of all analytes are presented in Table I. Six pairs of carbamates remain unresolved on Supersphere C_8-bonded silica. Studies on stationary phases with different selectivities, which are especially important for confirmatory purposes, are in progress.

In Fig. 1, the HPLC of a standard mixture of twelve N-methylcarbamates and ten of the sulphoxide and sulphone metabolites is shown. The separation time is less than 30 min. The additional equilibration at the initial mobile phase composition results in a total analysis time of 40 min. The N-methylcarbamates are detected via postcolumn reaction detection based on the hydrolysis of N-methylcarbamates to methylamine, which is reacted with OPA reagent to form a fluorescent isoindole. This derivative can be detected very selectively and sensitively. Detection limits calculated for a signal-to-noise ratio 3:1 are, on average, 100 pg, as can also be seen from Fig. 1.

TABLE I

RETENTION TIMES OF TWENTY N-METHYLCARBAMATE PESTICIDES AND TWELVE METABOLITES IN THE REVERSED-PHASE HPLC SYSTEM SUPERSPHERE RP-8 (4 μm) WITH AN ACETONITRILE–METHANOL–WATER GRADIENT

See Experimental for further details.

Peak No.	Carbamate/metabolite	Retention time (min)
1	Butocarboxim sulphoxide	5.90
2	Aldicarb sulphoxide	6.28
3	Butocarboxim sulphone	7.38
4	Aldicarb sulphone	7.85
5	Oxamyl	8.85
6	Methomyl	10.67
7	Ethiofencarb sulphoxide	12.76
8	Thiofanox sulphoxide	13.27
9	Ethiofencarb sulphone	13.81
	3-Hydroxycarbofuran	14.17
10	Methiocarb sulphoxide	14.86
	Tranid	15.00
	Dioxacarb	15.49
11	Thiofanox sulphone	15.63
12	Methiocarb sulphone	17.51
13	Butocarboxim	18.27
	3-Ketocarbofuran	18.85
14	Aldicarb	18.97
	Cloethocarb	21.15
15	Propoxur	21.47
	Bendiocarb	21.62
16	Carbofuran	21.75
17	Carbaryl	22.82
18	Ethiofencarb	23.27
	Thiofanox	23.46
	Isoprocarb	24.35
19	Landrin	24.35
20	Carbanolate	24.90
21	Methiocarb	26.49
	Fenobucarb	26.57
22	Promecarb	27.41
	Bufencarb	32.15

Catalytic solid-phase hydrolysis

Postcolumn hydrolysis of the N-methylcarbamates is usually performed by the addition of NaOH solution to the HPLC column effluent by means of a reagent pump. In order to simplify the postcolumn reaction, McGarvey [32] combined a KOH hydrolysis with the OPA reaction in a one-stage reaction, thereby eliminating one reagent pump. In spite of this elegant method and its potential advantages, applications to real sample analysis were not shown. Therefore, this approach may not have gained widespread acceptance.

In 1983, Nondek and co-workers [33,34] proposed the use of a solid-phase catalyst to replace the NaOH hydrolysis. Efficient hydrolysis was shown for six carbamates on Aminex-28, a strong anion exchanger. This principle was also adopted by She *et al.* [27] for the determination of carbaryl and by Jansen *et al.* [35], who miniaturized the postcolumn system for the narrow-bore HPLC of four N-methylcarbamates. Unfortunately, only brief applica-

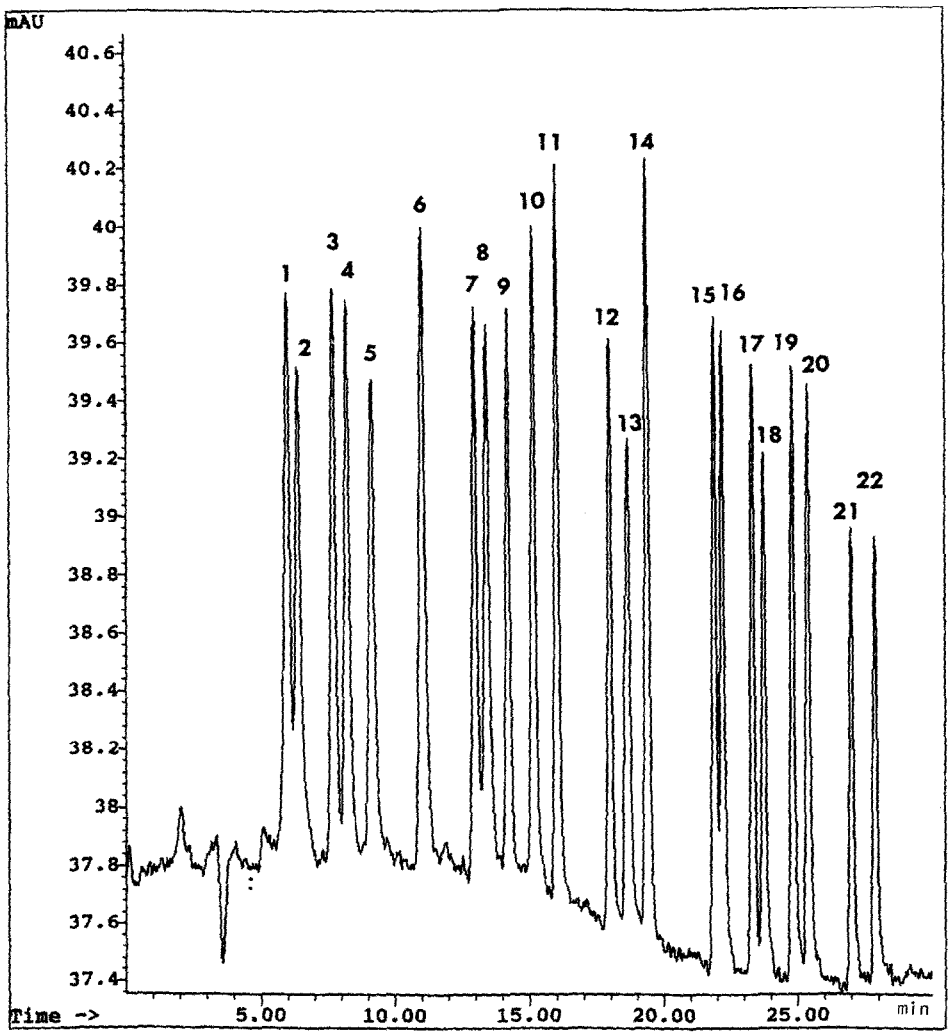

Fig. 1. HPLC of a standard mixture of twelve N-methylcarbamates and ten metabolites using Supersphere RP-8 (4 μm) with an acetonitrile–methanol–water gradient. Injected amounts: 1.0 ng of each carbamate. Fluorescence detection: excitation, 340 nm; emission, 445 nm. Peak numbers correspond to those in Table I. For further details, see Experimental.

tions for the analysis of water samples were described by these workers, which has hampered wider use by others. In 1990, de Kok *et al.* [6] examined various basic solid-phase materials and discovered that, apart from the strong anion-exchange material Aminex-27, the inexpensive, general-purpose magnesium oxide (MgO) has very favourable catalytic characteristics for the hydrolysis of N-methylcarbamates. The postcolumn solid-phase hydrolysis could be extended to include the whole group of more than 30 compounds tested [6]. In practice, the only problem with MgO is the relatively rapid blockage of the hydrolysis column due to the wide range of particle sizes from 100 μm down to 5 μm and the presence of fines. In routine crop analysis, the solid-phase hydrolysis principle has now been in use for more than four years in our laboratory [7], using the anion exchanger Aminex A-27, which has a narrow particle size range (15 ± 2 μm).

The major advantage of solid-phase hydrolysis compared with NaOH solution hydrolysis is the omission of an extra reagent pump, which means that no dilution of the HPLC column effluent takes place, thereby avoiding extra band broadening and retaining optimum resolution and peak sensitivity. Eliminating the NaOH hydrolysis also prevents unnecessary problems such as the occasional leaking of the reagent pump seals or blockage of the hydrolysis reaction capillary due to the build-up of crystallized NaOH reagent. In addition, daily preparation of NaOH reagent has become redundant. The lifetime of a well packed reactor column can be as long as 6 months, because the reaction is purely catalytic, which means that the solid phase is not consumed. Although ion exchangers are known for their incompatibility with drastic solvent changes, we have shown in a recent study [7] that gradient elution may be used without any problems at the prevalent reactor temperature of 120–140°C. Special care is required, however, if one changes to a very low flow-rate (0.05 ml/min), *e.g.*, overnight, to save solvent. The reactor column should first be allowed to cool nearly to room temperature, otherwise the ion-exchange resin will be deformed by the high temperature and blockage of the reactor column will occur.

Water sample analysis

Various research groups [17,19–21] use direct injection of large volumes of aqueous samples in their multi-residue methods for N-methylcarbamates. The AOAC recently adopted this technique for official first action [21]. The principle of large-volume injection is based on the on-column concentration of the analytes on an apolar stationary phase, which are injected dissolved in a purely aqueous phase. Subsequent gradient elution allows the normal separation of the mixture of analytes. Low determination limits in the range 0.2–4 μg/l can be obtained, especially in combination with sensitive postcolumn derivatization and fluorescence detection. Sample clean-up, other than filtration, is not required owing to the high selectivity of the HPLC detection system, and therefore virtually no recovery losses can occur. However, large injection volumes (> 500 μl) often broaden peaks and reduce the resolution, particularly for the early eluting peaks. Matrix peaks typically show up in the same part of the chromatogram, that is, matrix effects cannot be totally excluded.

In initial experiments, we also studied direct large-volume injection of surface water samples fortified with N-methylcarbamates. Volumes of 500 μl appeared to be the practical limit for good resolution of the polar sulphoxide and sulphone metabolites of butocarboxim and aldicarb, and therefore the ultimate determination limits on average were 0.2 μg/l. Keeping the EC tolerance limit (0.1 μg/l) in mind, further research had to be executed to achieve our aim of determination limits in the 0.02–0.03 μg/l range.

Liquid–liquid extraction

In the interest of efficiency and cost effectiveness in processing large numbers of environmental samples, it is highly desirable to determine as many pesticides as possible using a single extract, from which aliquots can be taken for subsequent analyses via GC, GC–mass spectrometry (MS), LC or LC–MS. For water samples, dichloromethane is widely accepted as an extraction solvent [36]. Typically, 1 l of water is extracted, the solvent evaporated and the residue dissolved in a small volume of a suitable solvent before chromatographic analysis. In our laboratory, crop sample analysis is performed according to Luke *et al.*'s method [37], followed by the recently developed, automated SPE clean-up on amino-bonded silica cartridges [7]. For SPE, the sample extract has to be evaporated and redissolved in dichloromethane. Hence, this clean-up procedure and on-line HPLC analysis seemed appropriate to combine with a dichloromethane extraction of water samples. The use of this combined method in initial experiments resulted in very clean chromatograms, even with highly polluted water samples from the rivers Rhine and Meuse. Surprisingly, omitting the SPE step also yielded satisfactory chromatograms, probably because of the selective postcolumn derivatization with fluorescence detection.

On the other hand, recoveries for the more polar carbamates after extraction of spiked surface water samples were insufficient. As a typical indicator of the polar carbamate metabolites, aldicarb sulphoxide was studied. When water volumes of 250–1000 ml were used, the recovery of aldicarb sulphoxide never exceeded 20%. This is caused by the unfa-

vourable partition coefficient in water–dichloromethane [7]. Bellar and Budde [36] also reported a recovery of 19% when a 1-l sample was extracted with three 60-ml portions of dichloromethane.

Higher extraction yields for (relatively) polar compounds can only be obtained by improving the ratio of organic to aqueous phase and/or increasing the number of (repetitive) extractions. As an example, in Table II the recovery results are shown for a 250-ml surface water sample fortified with sixteen N-methylcarbamates and/or metabolites at the 0.1 μg/l level, after three extractions with 100 ml of dichloromethane each. For aldicarb sulphoxide a 62% recovery was obtained. Most recoveries were well above 80% with relative standard deviations (R.S.D.s) below 10%, particularly for the parent compounds. However, the sulphoxides and sulphones were insufficiently extracted. The (extremely) low recoveries and high R.S.D.s for methiocarb sulphone and ethiofencarb sulphone can be ascribed to the fast degradation of these analytes in aqueous solution, which already starts when the samples are waiting for analysis in an autosampler queue. Acidification of the samples to pH 3 partly eliminates the problem. However, the overall result was unacceptable in terms of extraction recoveries, repeatability, sample preparation time and volumes of harzardous organic solvent used. Therefore, we decided to explore the potential of SPE for the concentration of N-methylcarbamates.

Liquid–solid extraction

We first investigated off-line SPE, because it can be applied in every laboratory without the necessity of purchasing expensive column-switching apparatus. In addition, it offers the opportunity to apply on-site field sampling and conservation of the analytes on the solid-phase material, which helps in stabilizing the labile carbamates. Finally, the validated HPLC method for N-methylcarbamate analysis can be used without any modification.

Off-line SPE has been used for the preconcentration of carbofuran [23], aldicarb [25] and carbaryl [24] and their respective metabolites. Sample volumes were 100, 10–20 and 1 ml, respectively, and determination limits were 0.4, 1 and 0.5 μg/l, respectively. Marvin *et al.* [30] concentrated 100-ml drinking water samples and obtained determination limits as low as 0.02–0.14 μg/l for carbaryl, propoxur and carbofuran, but this could only be achieved because of the relatively apolar nature of these three N-methylcarbamates. In their more recent study [31], breakthrough of the more polar aldicarb, aldicarb sulphoxide and aldicarb sulphone occured if water sample volumes exceeded 10 ml. Consequently, higher determination limits (7–11 μg/l) were obtained for these compounds. C_{18}-bonded [23,24,30,31] or C_8-bonded [25,31] silica phases were used in these studies. The sample volumes that can be preconcentrated on these apolar phases without breakthrough of the carbamates are strongly dependent on the polarity of the carbamate or metabolite studied. This means that the polar sulphoxides and sulphones are the critical compounds in SPE method development. We mainly focused our attention on these metabolites.

Most researchers use C_{18}-bonded silica cartridges to preconcentrate medium-polarity pesticides such as triazines and phenylurea herbicides from water. For more polar pesticides such as the N-methylcarbamates and, more so, their metabolites, this sorbent is not necessarily the best choice, as was shown by, *e.g.*, Lesage [25] and Chaput [26] in off-line and on-line SPE, respectively. They obtained significantly higher recoveries for aldicarb sulphoxide on C_8 than on C_{18}-bonded silica. This typical behaviour may be explained by selective sorption of the polar aldicarb sulphoxide on the free silanol groups of the silica, which are more accessible on the C_8 than the C_{18}-bonded material. To confirm these findings, we compared C_8- and C_{18}-bonded silica SPE cartridges from Analytichem that had not previously been tested for N-methylcarbamates.

The results obtained for real surface water samples from the IJsselmeer are given in Table II. The samples were fortified with a standard mixture of thirteen N-methylcarbamates and the ten most polar metabolites at two fortification levels (0.1 and 1.0 μg/l). When a sample volume of 50 ml was passed through the SPE cartridges, the recoveries for C_8- and C_{18}-bonded silica were in the range 34–108% (R.S.D. 0.7–7.5%) and 44–110% (R.S.D. 1.4–11.8%), respectively, at the 1.0 μg/l level. Recoveries at the 0.1 μg/l level were very similar, namely 32–112% and 40–109% for C_8- and C_{18}-bonded silica, respectively. Surprisingly, we did not notice higher recoveries on the C_8 phase for the more polar compounds (the first six peaks in the

TABLE II

AVERAGE PERCENT RECOVERIES [WITH R.S.D. (n = 5) IN PARENTHESES] OF N-METHYLCARBAMATES AND METABOLITES FROM SURFACE WATER, FORTIFIED AT 0.1 and 1.0 μg/l LEVELS, AFTER LIQUID–LIQUID OR SOLID-PHASE EXTRACTION

Carbamate/metabolite	Dichloromethane extraction (250 ml)[a]	Solid-phase extraction (0.1 μg/l) (50 ml)[a]			Solid-phase extraction (1.0 μg/l)			
					50 ml[a]			100 ml[a]
		C_8	C_{18}	C_{18}/OH	C_8	C_{18}	C_{18}/OH	C_{18}/OH
Butocarboxim sulphoxide		32	40	76 (3.9)	34 (6.1)	44 (4.9)	82 (3.9)	70 (4.8)
Aldicarb sulphoxide	62 (9.6)	37	45	83 (6.2)	42 (6.3)	55 (3.3)	93 (1.9)	78 (6.2)
Butocarboxim sulphone		41	46	80 (6.4)	47 (7.2)	49 (3.8)	88 (7.6)	42 (5.8)
Aldicarb sulphone		50	45	83 (6.5)	52 (5.7)	57 (4.1)	91 (8.5)	44 (3.7)
Oxamyl	82 (2.4)	83	83	89 (5.0)	86 (3.6)	87 (4.0)	111 (1.2)	74 (5.7)
Methomyl	91 (4.4)	58	65	81 (2.7)	60 (7.5)	72 (4.6)	90 (3.2)	46 (2.8)
Ethiofencarb sulphoxide	74 (9.4)	107	88	55 (4.8)	108 (3.2)	110 (10.2)	59 (10.3)	76 (4.7)
Thiofanox sulphoxide		110	91	42 (7.3)	105 (2.7)	107 (11.8)	38 (12.7)	54 (7.2)
Ethiofencarb sulphone	66 (24.2)	113	109	98 (4.5)	107 (2.9)	102 (3.1)	106 (2.4)	102 (2.8)
Methiocarb sulphoxide	89 (5.6)	111	106	80 (4.5)	108 (2.5)	101 (4.0)	77 (4.5)	95 (1.6)
Tranid		112	108	99 (3.2)	107 (2.2)	100 (2.1)	106 (1.4)	100 (1.8)
Thiofanox sulphone	91 (4.4)	112	109	102 (5.1)	105 (3.7)	102 (2.8)	106 (1.9)	101 (1.6)
Methiocarb sulphone	26 (26.0)	104	101	100 (2.6)	107 (3-3)	103 (2.6)	106 (1.9)	105 (2.7)
Butocarboxim		106	104	93 (1.0)	97 (1.9)	99 (1.7)	99 (1.4)	100 (1.3)
Aldicarb	69 (8.6)	101	99	95 (1.5)	100 (2.4)	99 (1.8)	103 (1.1)	99 (1.1)
Propoxur	89 (7.9)	102	103	98 (1.4)	102 (2.8)	100 (1.9)	104 (0.9)	100 (2.6)
Carbofuran	89 (7.8)	105	103	98 (1.8)	101 (3.4)	99 (3.2)	101 (0.5)	100 (2.7)
Carbaryl	89 (6.7)	104	104	104 (4.1)	102 (4.1)	102 (3.7)	102 (0.6)	99 (4.2)
Ethiofencarb		93	92	108 (3.1)	98 (3.1)	96 (3.6)	100 (2.7)	95 (3.7)
Carbonolate	82 (9.9)	97	97	89 (1.1)	99 (0.6)	97 (1.8)	100 (0.5)	98 (3.3)
Methiocarb	83 (8.4)	103	109	98 (3.0)	96 (2.9)	96 (3.4)	98 (2.1)	99 (2.7)
Promecarb	88 (5.7)	100	100	100 (2.6)	96 (0.7)	94 (1.4)	96 (2.2)	97 (2.4)
Bufencarb	82 (6.1)	95	99	96 (4.0)	93 (1.7)	87 (2.5)	92 (4.5)	97 (5.2)

[a] The volumes indicate the sample volumes extracted.

chromatogram). Apparently, the typical bonding chemistry and resulting surface characteristics of the C_8- and C_{18}-bonded phases from various manufacturers play an important role, which cannot easily be explained. Despite the incomplete recoveries due to breakthrough, the repeatability was encouraging.

In order to investigate further the influence of the free silanol groups of the solid-phase material on the recovery of polar carbamate metabolites, a new low-carbon C_{18}-bonded silica (C_{18}/OH), specially designed for polar metabolites of pharmaceuticals, was studied. Volumes of 50 ml of fortified water were concentrated on the C_{18}/OH phase. In general, recoveries at both fortification levels (76–111%) were better on this new sorbent than on the C_8- or C_{18}-bonded phases, as can be seen from Table II. The repeatability was very goood, with R.S.D.s of 0.5–8.5% and 1.0–7.3% for the 1.0 and 0.1 μg/l fortification levels, respectively. Only two exceptions

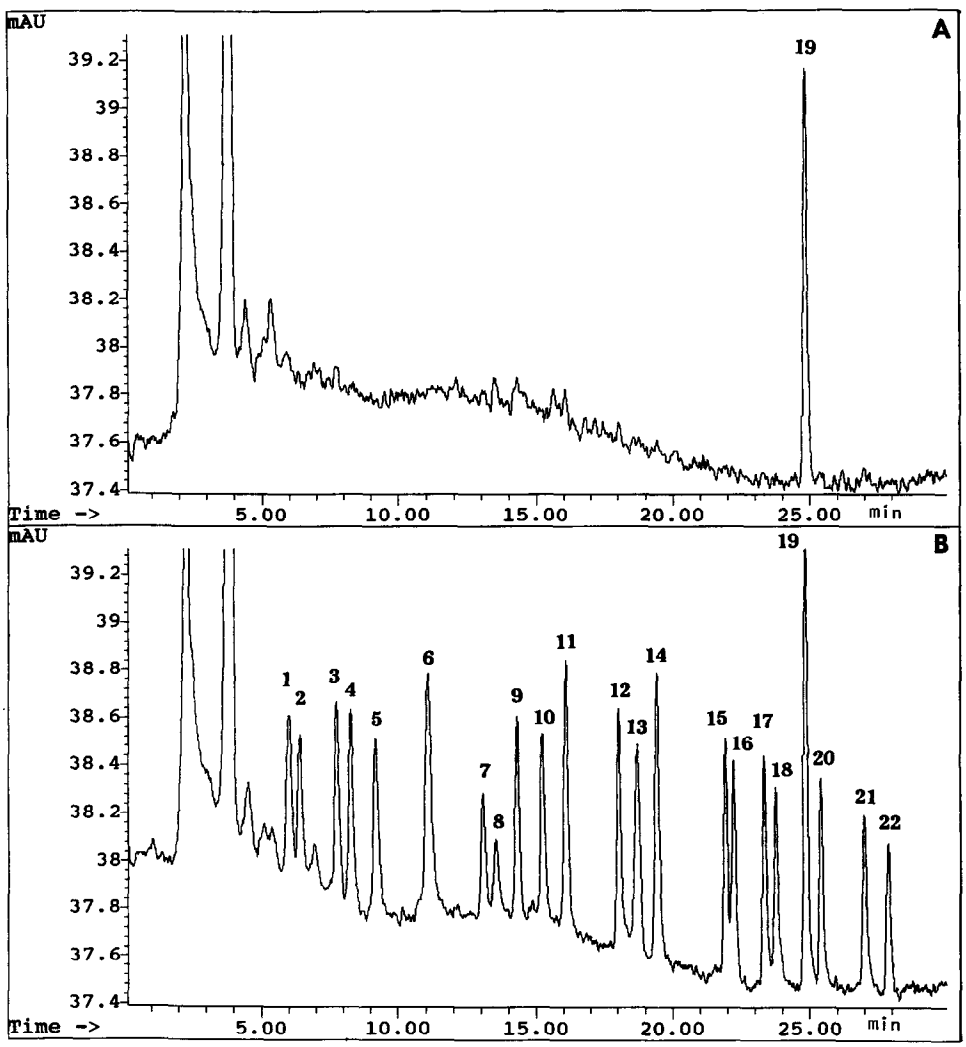

Fig. 2. HPLC of lake IJsselmeer surface water samples after SPE of 50 ml of (A) a blank water sample and (B) a water sample fortified with eleven N-methylcarbamates and ten metabolites at the 0.1 μg/l level on 500 mg C_{18}/OH cartridges. Landrin (peak 19; 1.0 ng injected) was used as an internal standard. In the case of 100% recovery, the injected amount (in 100 μl) of all other carbamates is 0.5 ng. Numbers above the peaks correspond to those given in Table I. For more details, see Experimental, Fig. 1 and text.

were noted, namely ethiofencarb sulphoxide and thiofanox sulphoxide, with recoveries in the 40–60% range with R.S.D.s slightly over 10% at the 1.0 μg/l level. Surprisingly, these two compounds were completely recovered on the other alkyl-bonded phases studied. This deviating behaviour is difficult to explain, because the recoveries were not significantly different at the two fortification levels or when a higher sample volume (100 ml) was concentrated. Increasing the sample volume from 50 to 100 ml resulted in breakthrough and hence, lower recoveries for the first six carbamates; however, the R.S.D.s remained very satisfactory. It is remarkable that the R.S.D.s are very low, irrespective of the sample volumes or the analyte concentrations. Even at the 0.1 μg/l level, which is a factor of *ca.* 3 above the determination limits, the R.S.D.s never exceed 10%.

It can be concluded that the C_{18}/OH phase has a special selectivity for the more polar carbamates oxamyl and methomyl and the sulphoxide and sulphone metabolites of aldicarb and butocarboxim, which can possibly be ascribed to the number and/or accessibility of the free silanol groups on the surface of the sorbent. In this context, one should note that the C_{18}/OH phase with its long alkyl chain concentrates the polar compounds better than does the C_8 phase with its shorter alkyl chain.

A chromatogram of an IJsselmeer surface water sample, fortified with eleven parent N-methylcarbamates and ten metabolites at a concentration of 0.1 μg/l and extracted on a 500-mg C_{18}/OH cartridge, is shown in Fig. 2. Landrin (peak 19), an older carbamate that has been taken out of production, was tentatively used as internal standard, and added after the acetonitrile elution step of the SPE procedure. A chromatogram of the corresponding blank surface water is included in Fig. 2 for comparison. The chromatograms are remarkably free from interfering peaks. The large peak with a retention time of *ca.* 3.5 min elutes well in front of the first carbamate metabolite. It originates from acetic acid, which is added to the water sample for conservation. Acidification (pH 3) of the water samples is important because many N-methylcarbamates and their metabolites are unstable under neutral or basic conditions.

With the optimized procedure, for all the N-methylcabamates listed in Table I, minimum detectable concentrations (signal-to-noise ratio = 3:1) were in the range 0.02–0.03 μg/l. These concentrations correspond to amounts injected into the HPLC system of 100–150 pg. This is surprisingly close to the detection limits of about 100 pg, obtained when standards are injected. Obviously, the clean-up and detection system are highly efficient and selective.

CONCLUSIONS

The multi-residue method described in this paper allows the detection of all twenty N-methylcarbamates and twelve polar metabolites studied in surface water down to the level of 20–30 ng/l using 50 ml of sample. Clean-up and preconcentration on selective C_{18}/OH SPE cartridges is a simple and rapid pretreatment step. The method is superior to conventional liquid–liquid extraction in terms of accuracy, precision and analysis time.

Off-line SPE allows the water samples to be concentrated on the cartridges at the collection site, which facilitates transportation and analyte conservation and is convenient when sophisticated laboratory facilities are not available. The method also lends itself to full automation with commercially available equipment such as the ASPEC (Gilson), as we have shown recently for crop samples [7]. A sample throughput of 36 samples per 24 h can then be achieved. Our current research is aimed at setting up such an automated system for water analysis.

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