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High-performance liquid chromatographic method for the analysis of aminocarb, mexacarbate and some of their N-methylcarbamate metabolites by post-column derivatization with fluorescence detection

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Abstract

A sensitive and selective high-performance liquid chromatographic method with post-column derivatization, using *o*-phthalaldehyde and 2-mercaptoethanol, is described for the analysis of aminocarb, mexacarbate and some of their carbamate metabolites. The separation system consisted of an RP-8 OS (10 μm) 20 cm × 4.6 mm I.D. column, an acetonitrile–water mobile system and a reactor for the hydrolysis of analytes from column effluents and fluorophore formation. The fluorophores were detected at 230 nm (excitation) and 418 nm (emission). The recoveries of the carbamates in spiked natural water at 2 and 20 ng/ml fortification levels ranged from 72.0 to 98.4% with relative standard deviations of 5.0 to 11.5%. The recoveries for spiked forest soil at 20 and 200 ng/g fortification levels ranged from 74.1 to 97.6% with relative standard deviations of 5.8 to 10.7%. Limits of detection and quantification for all the analytes were 0.1 and 0.4 ng in water and soil, respectively.

1. Introduction

Aminocarb (4-dimethylamino-3-methylphenyl N-methylcarbamate, trade name Matacil; Mobay, Kansas City, MO, USA) and mexacarbate (4-dimethylamino-3,5-xylyl N-methylcarbamate, trade name Zectran; Union Carbide Agricultural Products, Research Triangle Park, NC, USA) are highly effective broad-spectrum commercial insecticides derived from carbamic acid. Aminocarb has been one of the principal insecticides used aerially in eastern Canada since 1975 against the spruce budworm, *Choristoneura fumiferana* (Clemens), a destructive defoliator of conifers. Mexacarbate has been used off and on in experimental aerial spray programs since 1969. The two carbamates degrade initially with the carbamate moiety intact when applied to foliar surfaces [1]. Some of these degradation products, such as 4-methylamino, 4-methylformamido, 4-amino and 4-formamido analogues, like the parent materials, are toxic [1–3] causing concern to regulatory and scientific communities.

To evaluate the potential hazard from the use of aminocarb and mexacarbate, it is necessary to know their fate in different forest matrices. This requires sensitive, efficient and reliable analytical methods to determine the parent materials and

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their degradation products. Numerous gas–liquid (GLC) and high-pressure liquid chromatographic (HPLC) methods have been described for the analysis of carbamate residues in a variety of agricultural matrices [4–6]. However, the preferred method for the analysis of aminocarb and mexacarbate from forestry matrices is by GLC [7] and for mexacarbate and its metabolites by HPLC–UV detection [8]. Drawbacks of the GLC method are the laborious cleanup and partition procedures involved, while disadvantages of the HPLC method include its insensitivity for all the analyte–matrix combinations studied. Moye *et al.* [9] introduced HPLC–fluorescence for the detection of fluorophores following post-column derivatization (PCD). Krause [10–14] refined the method and Engelhardt and Lillig [15] optimized the procedure, making it a sensitive and selective approach with widespread acceptance for the analysis of N-methylcarbamate residues.

The purpose of this study was to extend these techniques to develop an expeditious and sensitive method to quantify aminocarb, mexacarbate and their carbamate metabolites that are likely to be formed (Table 1) in natural waters and forest soil, which are the major receptors of aerially sprayed insecticides. The analytes, after elution through the HPLC system, were hydrolysed by aqueous sodium hydroxide, then the primary amines were derivatized forming the highly fluorescent isoindole derivative (Fig. 1), using *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (MCE), and their fluorescence was measured by the on-line detector (Fig. 2). The method was used to examine its applicability to quantify the analytes in natural water and forest soil after necessary fortification, extraction, cleanup and derivatization steps.

2. Experimental

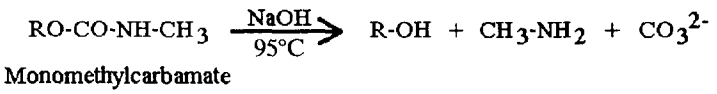
2.1. Reagents

Analytical-grade standards of aminocarb and four of its metabolites were supplied by Mobay, while mexacarbate and four of its metabolites were supplied by Union Carbide Agricultural Products. The common and chemical names

Table 1
List of aminocarb and mexacarbate and their carbamate metabolites used in the study

Designation	Chemical name	Abbreviation
Aminocarb	4-Dimethylamino-3-methylphenyl N-methylcarbamate	A
Methylformamido aminocarb	4-Methylformamido-3-methylphenyl N-methylcarbamate	MFA
Methylamino aminocarb	4-Methylamino-3-methylphenyl N-methylcarbamate	MA
Formamido aminocarb	4-Formamido-3-methylphenyl N-methylcarbamate	FA
Amino aminocarb	4-Amino-3-methylphenyl N-methylcarbamate	AA
Mexacarbate	4-Dimethylamino-3,5-xylyl N-methylcarbamate	M
Methylformamido mexacarbate	4-Methylformamido-3,5-xylyl N-methylcarbamate	MFM
Methylamino mexacarbate	4-Methylamino-3,5-xylyl N-methylcarbamate	MAM
Formamido mexacarbate	4-Formamido-3,5-xylyl N-methylcarbamate	FM
Amino mexacarbate	4-Amino-3,5-xylyl N-methylcarbamate	AM

HYDROLYSIS



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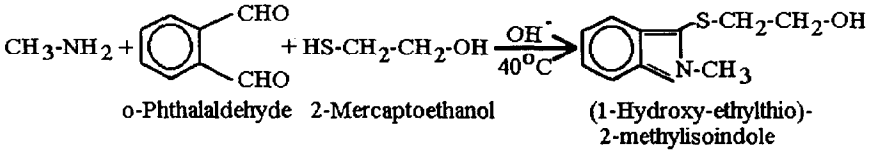

Fig. 1. Reaction scheme of post-column derivatization.

(IUPAC) and the abbreviations used for each are listed in Table 1. All analytical standards were greater than 98.5% pure and stored at −20°C in sealed vials until use.

Methanol, ethyl acetate, *n*-hexane, acetonitrile and water were HPLC grade and obtained from J.T. Baker, through Baxter (Canlab Division), Mississauga, Canada. Alumina (activity 1, type WN-6 neutral) was from Sigma, Mississauga, Canada. OPA and MCE were obtained from BDH, Toronto, Canada. Analytical-grade sodium hydroxide, sodium sulphate (anhydrous, granular) and boric acid were obtained from Fisher Scientific, Don Mills, Canada.

Borate buffer (pH 10.4) was prepared by dissolving 12.4 g of boric acid in 1 l of water and adjusting the pH to 10.4 by adding 2 *M* NaOH. The derivatization reagent was prepared first by dissolving 160 mg OPA in 2 ml methanol and then adding 0.10 ml of MCE followed by 200 ml of borate buffer. The solution was mixed well and filtered through a 0.45-μm membrane filter (product No. 66068, Gelman Sciences, Rexdale, Canada) and stored at 4°C. The hydrolysis reagent (0.05 *M* NaOH) was prepared by dissolving 2.0 g of sodium hydroxide in 1 l of solution, filtering through a 0.45-μm membrane filter and degassing with helium. Natural stream water (pH 6.2, hardness 16 μg $CaCO_3$/ml, alkalinity 10.2 μg $CaCO_3$/ml, conductivity 2.7 μS) was col-

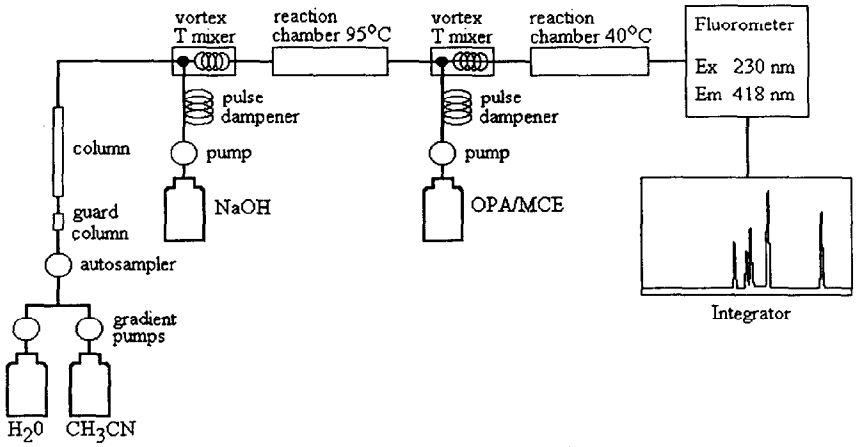

Fig. 2. HPLC post-column fluorometric system.

lected from the Icewater Creek near Searchmont, Ontario, Canada, filtered through a 5-μm filter and stored at 4°C until use. Forest soil (organic matter 3.9%, sand 51%, silt 42%, clay 7% and pH 5.91) was collected from the same area. It was sieved (2 mm opening) to remove stones, root pieces, etc., and stored in glass jars at −20°C until use.

Standard stock solutions (50 ml) of each analyte noted in Table 1 were prepared separately in amber-coloured volumetric flasks by dissolving 10 mg of the material in acetonitrile. Standard working solutions were prepared by diluting aliquots of each stock solution with acetonitrile to give concentrations ranging from 0.5 to 500 ng/ml. Similarly, mixed standards of the parent compounds, aminocarb and mexacarbate, together with their four respective metabolites, were prepared separately by mixing aliquot quantities of the individual standard stock solutions, so that the concentrations of each analyte in the mixed standards ranged from 0.5 to 500 ng/ml. Unless used immediately, the solutions were stored at −20°C.

2.2. Apparatus

A Hewlett-Packard (HP) 1084B HPLC system was interfaced with a HP 79842A variable-volume injector and autosampler (Model 79842). The instrument also incorporated an automatic degassing system and two dual-head reciprocating solvent-delivery systems controlled by a solvent flow programmer to give stable, reproducible flows. A prepacked HP RP-8 OS (10 μm) stainless-steel separation column (20 cm × 4.6 mm I.D.) was used in conjunction with a HP RP-8 guard column (3 cm × 4.6 mm I.D., 10 μm). The column temperature was kept at 30°C to maintain retention time reproducibility. The fluorescence detector was a Kratos (Ramsey, NJ, USA) FS970 LC fluorometer equipped with a 10-μl flow-cell and automatic overload reset (FSA 986) with variable excitation wavelengths (GM 970 monochromator) and fixed-wavelength emission filters. The derivatization was done in a Kratos URS 051 post-column reaction system (PCRS) equipped with two reagent pumps, hydrolysis and reaction coils and a URA 200 temperature controller. The detector signal was recorded and processed with a HP data module (Model 79850B), which provided the chromatogram, area, retention time, etc., for each peak. Additional operating parameters were: mobile phase: acetonitrile–water; flow-rate: 1.0 ml/min; injection volume: 100 μl; run time: 60 min; gradient [% (v/v) acetonitrile]: 0 min 0%, 25 min 30%, 35 min 50%, 45 min 50%, 55 min 0%; derivatization: hydrolysis: 0.5 ml NaOH/min, hydrolysis coil 1 ml, temperature 95°C, fluorophore: 0.3 ml OPA–MCE/min, reaction coil 0.5 ml, temperature 40°C; detection: excitation 230 nm, emission 418 nm.

2.3. Evaluation

To evaluate sensitivity, standard solutions of aminocarb and mexacarbate containing their respective metabolites were injected separately several times ($n > 6$) to obtain reproducible peak area measurements under the PCD conditions mentioned previously. Individual standards were used for the FA and AA metabolites of aminocarb, because of poor chromatographic separation (see section 3). The relative standard deviation (R.S.D.) between injections ranged from 1.1 to 4.1% depending on the type of analyte. Similarly, replicate analysis of mixed standards (except FA and AA) at four-day intervals gave good reproducibility (average R.S.D. < 4%).

2.4. Recovery studies from natural water

To study the recovery of the analytes, 100-ml aliquots of natural stream water were fortified in triplicate with mixed standards of aminocarb and mexacarbate, and their respective metabolites, to yield concentrations of 2 and 20 ng/ml of each analyte. Due to co-elution of FA and AA on the HPLC column, water samples were fortified with these metabolites separately to assess the recovery efficiencies for these compounds. Each fortified water sample was serially extracted

using 3 × 40 ml of dichloromethane. The organic layer was dried with anhydrous sodium sulphate and flash-evaporated to dryness at 30°C. The residue, after flash-evaporation, was dissolved in 30 ml of acetonitrile and partitioned twice with 10 ml of hexane each time. The polar layer was flash-evaporated to dryness, dissolved in ethyl acetate, transferred to a graduated centrifuge tube and the volume was adjusted to 10 ml by evaporation under a stream of pure, dry nitrogen. A 1-ml volume of the crude extract was loaded onto an alumina minicolumn (1 g Al_2O_3 sandwiched between 0.5-cm thick layers of anhydrous sodium sulphate in a glass wool-plugged disposable Pasteur pipette, 14.5 cm × 7.5 mm I.D., preconditioned with 10 ml of ethyl acetate). Commercially available solid-phase extraction cartridges containing Al_2O_3 (PrepSep extraction columns, Fisher Scientific, Unionville, Canada) were found to be equally suitable for the cleanup, except for the high cost involved in analyzing large numbers of samples, normally expected following a spray application. The analytes were eluted with 15 ml of ethyl acetate, with the exception of the aldehyde moieties (MFA, FA, MFM and FM), which required an additional 10 ml of polar-modified eluent containing 10% (v/v) methanol. The eluates were evaporated to dryness and recovered in 1 ml of acetonitrile for HPLC analysis. Recovery levels of the analytes in water were determined by injecting aliquots of analytical solutions and comparing the peak areas with those of the standard solutions.

2.5. Recovery studies from forest soil

To study the recoveries of the analytes in soil, 10-g aliquots were fortified in triplicate to give 20 and 200 ng/g of each analyte. Each sample was extracted (Sorval Omni-Mixer; Ivan Sorval, Norwalk, CT, USA) thrice using 50 ml ethyl acetate each time. After passing through a column of anhydrous sodium sulfate (3 × 2.5 cm), the ethyl acetate was flash-evaporated to dryness at 30°C. The remaining cleanup and recovery determinations were similar to water.

3. Results and discussion

3.1. Optimization of chromatographic response

Attempts to alter the excitation and emission wavelengths to optimize sensitivity were not successful. In particular, longer excitation wavelengths resulted in considerable sensitivity reductions for all of the analytes. The use of a methanol–water gradient system did not distinctly resolve all of the peaks and the sensitivity was lower compared to the acetonitrile–water mobile phase. Also, efforts to optimize the post-column reaction conditions by adjusting the molarity of sodium hydroxide used in hydrolysis, temperature of the reactor etc. did not enhance sensitivity or resolution of the compounds.

3.2. Linearity of the detector and detection limit

Linearity of the fluorescence detector was determined by 100-μl injections of serially diluted standards of both insecticides and their metabolites in the range of 0.5 to 500 ng/ml. Peak areas were integrated electronically and plotted against concentration. Regression analysis of the data indicated linearity of the detector from 0.1 to 50 ng for all the analytes and correlation coefficients ranged from 0.981 to 1.00 depending on the type of analyte. These results illustrate that PCD with fluorescence detection is a very sensitive technique and can be used to detect and quantify the analytes in Table 1.

3.3. Chromatograms of the mixed standards

Figs. 3 and 4 illustrate the chromatographic separations obtained respectively for the mixed standards of aminocarb and its four metabolites and mexacarbate and its four metabolites. Mean retention times (min) of each individual compound (six determinations) are given in the caption of each figure. The variation in retention time was less than 2% for all the analytes. The separation of FA and AA (Fig. 3) was not possible under any of the experimental conditions tested. Altering experimental variables, such as mobile phase composition, solvent sys-

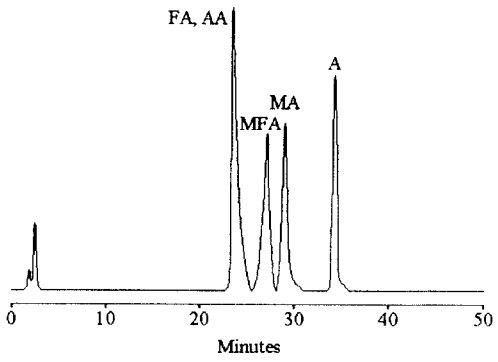

Fig. 3. Sample chromatogram of aminocarb and its four metabolites (5 ng/injection) with retention times (min): A = 34.34; MFA = 27.06; MA = 29.07; FA, AA = 23.43.

tems with varying solvent strength, column type, length and packing, flow-rates, etc., did not result in the disengagement of FA and AA bands. It is likely that the use of different fluorophore systems could result in improved resolution. The chromatographic separation of mexacarbate and its metabolites (Fig. 4) is satisfactory, the peaks are sharp and well defined. The peaks of AM and MFM are not fully resolved, but good integration parameters (properly set peak width and peak threshold values) allowed satisfactory quantitation of the two analytes.

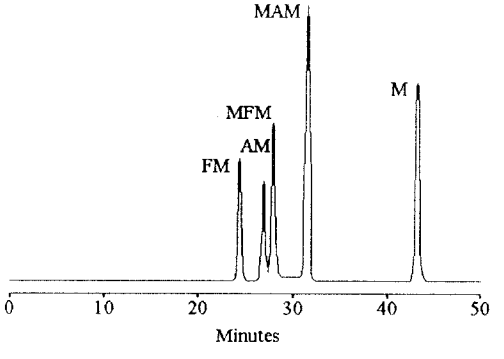

Fig. 4. Sample chromatogram of mexacarbate and its four metabolites (5 ng/injection) with retention times (min): M = 43.31; MFM = 28.04; MAM = 31.62; FM = 24.43; AM = 27.01.

3.4. Recoveries of carbamates from water and soil

Recoveries of the carbamates obtained from natural water at 2 and 20 ng/ml fortification levels and forest soil at 20 and 200 ng/g are given in Table 2 along with their S.D. and R.S.D. values. Corresponding chromatograms are given in Figs. 5 (water blank after cleanup), 6 (aminocarb and its metabolites in water at 20 ng/ml fortification level), 7 (mexacarbate and its metabolites in water at 20 ng/ml), 8 (soil blank after cleanup), 9 (aminocarb and its metabolites in soil at 200 ng/g) and 10 (mexacarbate and its metabolites in soil at 200 ng/g). Recoveries of the two parent insecticides, A and M, were above 93% at both fortification levels with R.S.D.s less than 6.5% (Table 2). However, compared to other analytes, the recoveries of the four aldehydes at both fortification levels in water and soil were low, especially the FA and FM which averaged only $77.8 \pm 5.6\%$. The low recoveries in water could be due to their high polarity and enhanced water solubility, preventing quantitative partition from aqueous to organic phase. Generally, from the data in Table 2, it is apparent that the recoveries of the analytes from water and soil are slightly higher at the higher fortification level, but the ranges in R.S.D. (5.0 to 11.5%) were nearly the same in both cases. The limit of detection (LOD) for each analyte, calculated as three times the S.D. of the blank response [16], was in the order of 0.1 ng. Limit of quantification (LOQ) ($10 \times$ S.D.) was determined to be 0.4 ng for all the analytes.

The Al_2O_3 minicolumn method provided adequate cleanup for natural water and forest soil by removing the bulk of the coextracted impurities. The chromatograms of the blank water and soil samples (Figs. 5 and 8) exhibit no peaks which would interfere with the analysis of the carbamate analytes. Figs. 6 and 9 show the typical chromatograms obtained for aminocarb and its metabolites in fortified samples of water and soil respectively. Similar chromatograms for mexacarbate and its metabolites are given in Figs. 7 and 10. Like the blanks, they are clean

Table 2
Average recoveries ($n = 3$) of aminocarb and mexacarbate and their carbamate metabolites[a] from natural water and forest soil

Carbamate	Recovery ± S.D. (R.S.D., %)			
	Fortification			
	Natural water		Forest soil	
	2 ng/ml	20 ng/ml	20 ng/g	200 ng/g
A	95.1 ± 5.9 (6.2)	98.4 ± 5.2 (5.3)	95.7 ± 6.1 (6.4)	97.6 ± 5.7 (5.8)
MFA	87.8 ± 6.7 (7.6)	91.2 ± 6.4 (7.0)	85.2 ± 7.1 (8.3)	89.9 ± 6.8 (7.6)
MA	91.4 ± 6.1 (6.7)	93.7 ± 5.7 (6.1)	93.6 ± 6.9 (7.4)	95.3 ± 6.1 (6.4)
FA	73.2 ± 8.4 (11.5)	77.6 ± 8.3 (10.7)	81.1 ± 8.7 (10.7)	84.7 ± 7.9 (9.3)
AA	83.6 ± 7.8 (9.3)	87.2 ± 7.7 (8.8)	78.3 ± 8.2 (10.5)	84.8 ± 7.8 (9.2)
M	94.2 ± 5.3 (5.6)	98.2 ± 4.9 (5.0)	93.6 ± 6.0 (6.4)	96.2 ± 5.7 (5.9)
MFM	82.7 ± 6.6 (8.0)	85.6 ± 6.6 (7.7)	81.4 ± 7.8 (9.6)	85.1 ± 7.2 (8.5)
MAM	92.8 ± 6.3 (6.8)	94.6 ± 6.1 (6.4)	93.3 ± 6.5 (7.0)	94.9 ± 6.3 (6.6)
FM	72.0 ± 7.9 (11.0)	75.1 ± 8.2 (10.9)	77.6 ± 8.3 (10.7)	81.0 ± 7.5 (9.3)
AM	74.7 ± 7.5 (10.0)	84.6 ± 7.1 (8.4)	74.1 ± 7.2 (9.7)	82.4 ± 6.3 (7.6)

[a] FA and AA were fortified separately.

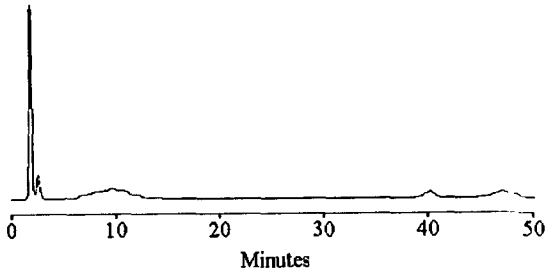

Fig. 5. Chromatogram of natural water blank after column cleanup.

and free from interfering compounds. Any minor matrix interference did not affect the analysis. The Nuchar–Celite mixture used by others [4,12] in the column cleanup was tried for the water and soil samples and found to be unsatisfactory, due to low recovery levels, especially for the aldehydes, and also because of interference peaks due to coextractive impurities.

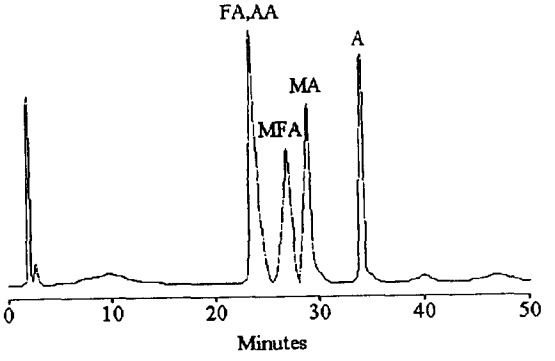

Fig. 6. Chromatogram of water fortified with aminocarb and its metabolites each at 20 ng/ml (100 μl/injection).

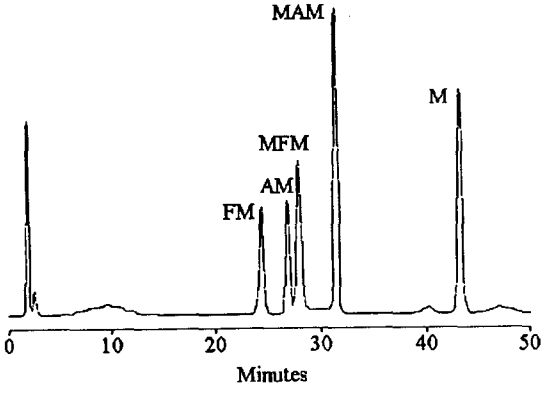

Fig. 7. Chromatogram of water fortified with mexacarbate and its metabolites each at 20 ng/ml (100 μl/injection).

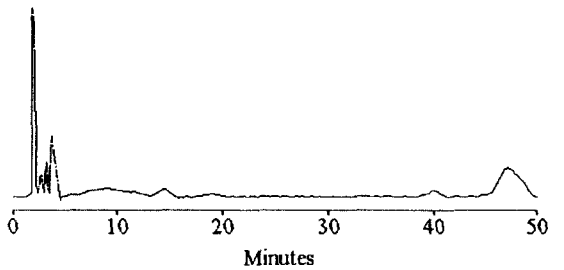

Fig. 8. Chromatogram of soil blank after column cleanup.

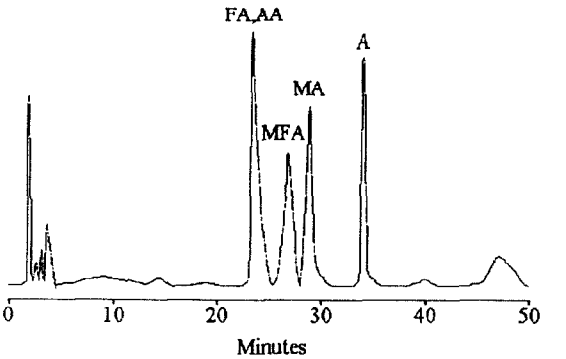

Fig. 9. Chromatogram of soil fortified with aminocarb and its metabolites each at 200 ng/g (100 μl/injection).

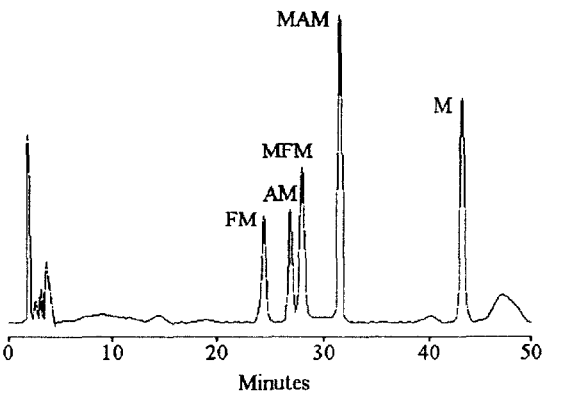

Fig. 10. Chromatogram of soil fortified with mexacarbate and its metabolites each at 200 ng/g (100 μl/injection).

4. Conclusions

The HPLC–PCRS method reported in this paper is suitable for isolating and quantifying low levels of aminocarb and mexacarbate and their carbamate metabolites in natural water and forest soil with good reproducibility. The method is straightforward and combines high selectivity due to pre-run chromatographic separation and high sensitivity of fluorometric detection. The on-line coupling of separation, derivatization and detection simplifies the analytical scheme thus making the method effective for routine use in situations where a lot of samples will be analyzed. With the development of suitable extraction and cleanup techniques, the method could be extended to analyze the residues of monoalkyl carbamates in different environmental matrices, such as conifer needles, forest litter, etc.

5. References

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