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Determination of Aminocarb in Water by High Performance Liquid Chromatography

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Aminocarb (4-dimethylamino-3-methylphenyl N-methylcarbamate) is a carbamate insecticide that has been used extensively in eastern Canada to control forest infestation by the spruce budworm (Choristoneura fumiferana). In such spraying operations, basic requirements for sensitive, efficient and reliable methodologies exist to account for the fate of the biocides. There have been relatively few residue methods published to date concerning aminocarb.

The direct analysis of carbamate insecticides by gas liquid chromatography (GLC) is deemed difficult because of on-column instability at normal operating temperatures. However, WHEELER et al. (1974) reported the direct GLC analysis of some N-methylcarbamates on an OV-17 column. They observed approximately 8% decomposition for aminocarb at 180°C and the stability of other carbamates was shown to vary according to column type and temperatures used.

Some analysts prefer derivitisation prior to GLC especially when general screening of a group of carbamates is required. LAWRENCE (1976) found the use of a catalyst such as trimethylamine substantially increased the rate of heptaflurobutyrylation and trifluoroacetylation of aminocarb and other carbamates. This is substantially less time consuming compared to the otherwise rigorous method. The derivatives are apparently stable for only a few days, however. A superior method is that based on work by HOLDEN (1973) in which carbamates are transformed to their 2-4-dinitrophenyl ether derivatives for determination by electron capture GLC. The main draw-back is that the N-methylcarbamates containing a p-dimethylamino group, notably aminocarb and mexacarbate are not recovered quantitatively from the clean-up step.

In view of the inherent difficulties with GLC analysis, high performance liquid chromatography (HPLC) is a promising alternative. LAWRENCE (1977) investigated the direct analysis of some carbamates including aminocarb in food, by normal phase HPLC with fixed wavelength ultraviolet detection. SPARACINO et al. (1976) reported the HPLC of some thirty carbamates excluding aminocarb by both normal and reverse phase modes and MOYE et al. (1977) described the dynamic post-column fluorogenic labelling of N-methylcarbamates with o-phthaldehyde.

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In this paper we describe the analysis of aminocarb by reverse phase HPLC using UV absorbance in series with fluorescence detection. A pre-concentration technique using Amberlite(R) XAD-4 resin for the analysis of rain water is also elaborated.

MATERIALS AND METHODS

Chemicals and Equipment - The acetonitrile used was HPLC grade and all other solvents were pesticide grade. Reagents and other materials were solvent washed and heated where necessary. Standard materials were obtained from Mobay Chemical Corporation Ltd. The Amberlite(R) XAD-4 resin (20-50 mesh) was cleaned by Soxhlet according to REES et al. (1979). The glass column (30 x 2.2 cm i.d.) was packed (to a height of 12 cm) according to the procedure reported by MALLET et al. (1978) and regenerated after use by flushing with 100 mL acetone followed by 200 mL distilled water.

A Spectra Physics' Model 8000 HPLC equipped as follows was used; automatic sampler, 10µL loop injector, Model 230 fixed wavelength UV absorbance (254 nm) detector connected in series with a Schoeffel FS-970 fluorescence detector, (λEX = 248 nm, emission filter with cut off > 370 nm) and data system with printer/plotter. A mobile phase of 40% acetonitrile in phosphate buffer (pH 6.9, 0.01 M) was run at a flow rate of 2.0 mL/min. through a bonded-phase Lichrosorb RP-8 (10 µ) column (25 x 0.46 cm i.d.) unless specified otherwise. Working standard solutions of aminocarb were prepared at 5.0 ng/µL and 1.0 ng/µL in acetonitrile to calibrate the two detectors respectively.

Extraction and Analysis - The water sample was poured through the resin column under gravity flow and successively eluted with three 40 mL portions of ethyl acetate. The combined portions were dried by adding 5 - 10 g Na_2SO_4 and then passed through a Na_2SO_4 column. The extract was concentrated to approximately 5 - 10 mL on a rotary evaporator at 35°C and solvent replaced with acetonitrile. The final evaporation was then continued in a 10 mL concentrator tube with adaptor and then made-up to volume in the appropriate flask for subsequent HPLC analysis.

Partitioning - When the sample extract required cleaning, acid-base partitioning was performed by adding the concentrated extract to 300 mL of distilled water and adjusting to pH 3 with 50% H_2SO_4. Repeating the process twice, 50 mL of ethyl acetate was added and shaken, the layers separated and organic phase discarded. The pH of the aqueous phase was then adjusted to 7.0 with 5 N and 0.5 N NaOH respectively, extracted thrice with 50 mL portions of ethyl acetate, dried and reduced in the same manner as described above.

RESULTS AND DISCUSSION

Some degradation products of aminocarb were included in the study to assess whether they would interfere with the determination

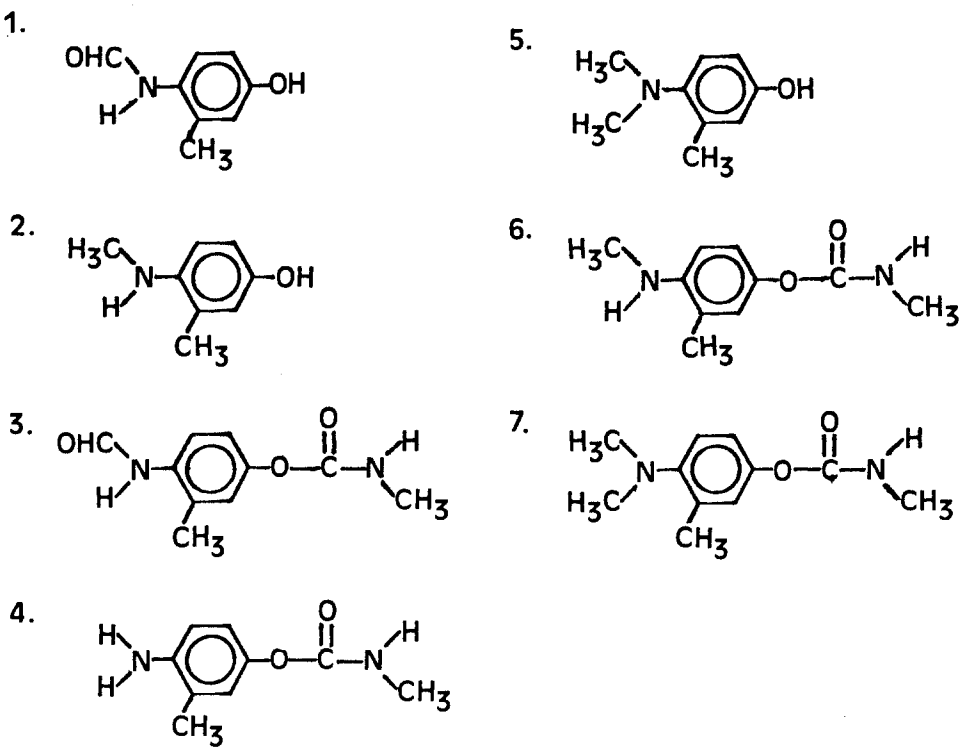

Fig. 1. Structures of aminocarb and some derivatives:
1. 4-formamido-3-methylphenol 2. 4-methylamino-3-methylphenol
3. 4-formamido-3-methylphenyl N-methylcarbamate 4. 4-amino-3-methylphenyl N-methylcarbamate 5. 4-dimethylamino-3-methylphenol
6. 4-methylamino-3-methylphenyl N-methylcarbamate 7. 4-dimethylamino-3-methylphenyl N-methylcarbamate (aminocarb).

of the parent compound and to show whether their identification and quantitation was feasible. The structure of aminocarb and analogous compounds are shown in Figure 1. Compounds 3, 4 and 6 have been tentatively identified elsewhere (KUHR et al. 1976) as animal and plant metabolites and photolytic products. They apparently retain considerable toxicity and some, namely 4 and 6, are even more potent than the parent compound itself. There is no mention of the phenolic derivatives 1, 2 and 5 elsewhere in the literature in terms of being potential degradation products but hydrolysis could be a mechanism for further breakdown and these would then become likely intermediates.

A fluorescence trace of aminocarb and its respective phenolic product is presented in Figure 2. Optimum excitation found was at 248 nm with a 7-54 narrow band-pass filter in front of the lamp and a cut-off filter greater than 370 nm selected for emission. These spectral properties fall in fairly close agreement with

those measured by ADDISON et al. (1977) considering the nature of the solvent used. Optimum fluorescence properties of the other compounds were not determined and the relatively lower responses noted for compounds 2, 4 and 6 (Figure 3) and negative responses for 1 and 3 are probably due to improper wavelength/filter selection and some quenching by the N-formyl functional group.

Figure 4 is the chromatogram of the same mixture with the UV detector at 254 nm. Compounds 1 and 3 as expected gave a good response on this detector but peak 3 completely overlapped with 4. Differentiation is however possible by comparing both UV and fluorescence traces, noting that compound 3 lacks response on the latter detector. The shoulders on peak 1 and peak 3 & 4 are due to impurities in the standard materials. Response for all compounds was found generally to be lower at 280 nm than at 254 nm. The unmarked peaks at the beginning of the fluorescence chromatograms in Figures 2 & 3 are due to sample solvent. The peaks prior to peak 1 in Figure 4 are also the result of the sample solvent and are attributed to the so-called refractive index response common to absorption detectors.

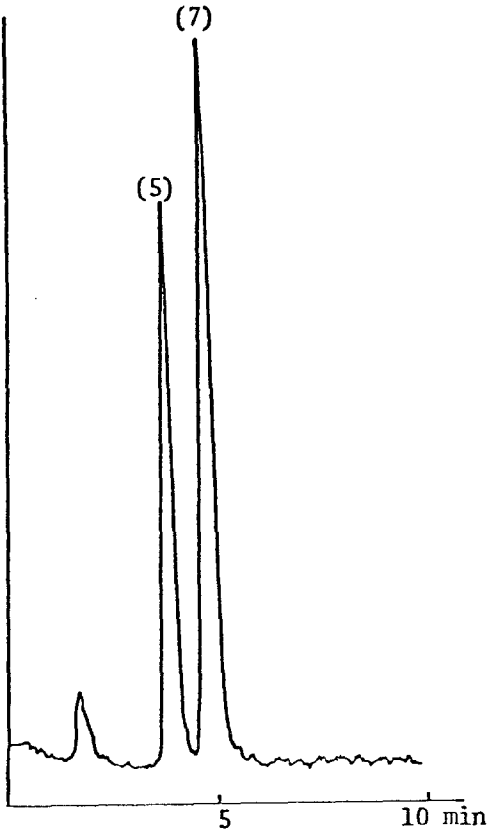

Fig. 2. HPLC of aminocarb (7) and phenolic derivative (5) by fluorescence detection. Conditions: solvent system = acetonitrile: buffer (40:60). Concentration = 1.0 ng/uL each. Sensitivity = 0.2 uAmp.FS. Column = RP-8.

Other columns such as the Lichrosorb RP-8 (10 µ) and HC-ODS/ Sil-X did not give improved resolution of the more polar compounds and resulted in increased run times. Furthermore the latter was unsatisfactory because of its greater lipophilicity. On the other hand the RP-8 compared to the RP-2 column works best with the conditions stipulated in Figure 2 for applications regarding the parent compound and its phenolic product which are comparatively more lipophylic. In other words, sloping baseline at the start of the run emanating from polar co-extractives and partial overlapping was often found to interfere with peaks of interest on the RP-2 column.

It's noteworthy that some transformation of the aminocarb phenol kept in acetonitrile (100 ng/µL) in a clear flask was

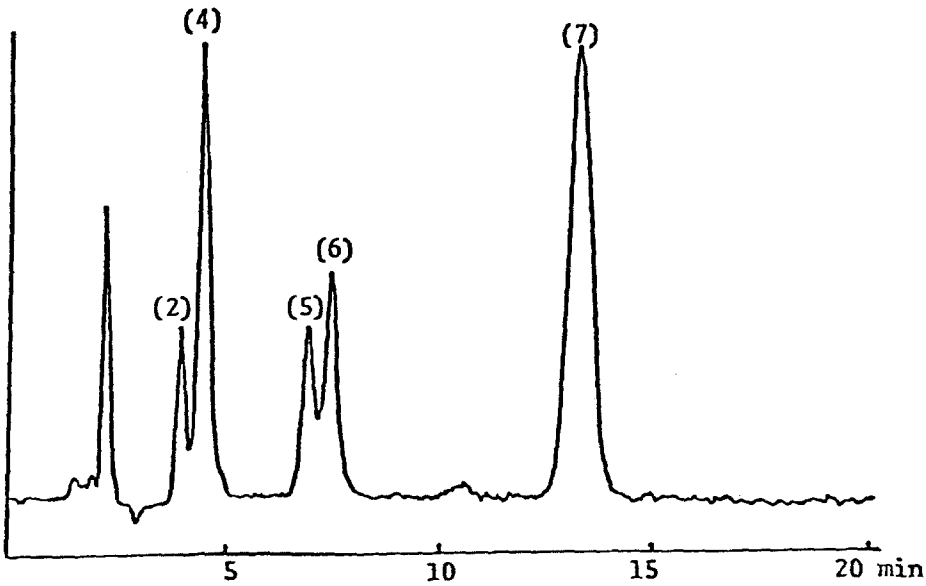

Fig. 3. HPLC of aminocarb and derivatives by fluorescence detection. Conditions: solvent system = acetonitrile: buffer (15:85). Concentrations; (2),(4),(6) = 10.0 ng/uL; (5),(7) = 1.0 ng/uL. Sensitivity = 0.1 uAmp.FS. Column = RP-2.

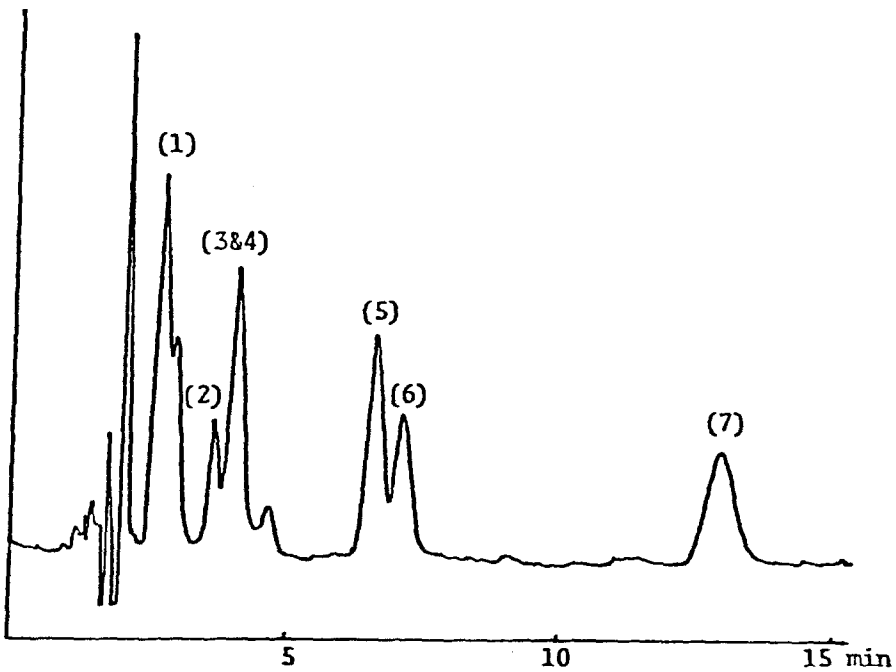

Fig. 4. HPLC of aminocarb and derivatives by UV-absorption at 254 nm. Conditions: solvent system = acetonitrile: buffer (15:85). Concentrations; (1),(2) = 10 ng/uL; (3),(4),(7) = 20 ng/uL; (5) = 30 ng/uL; (6) = 40 ng/uL. Sensitivity = 0.02 AUFS. Column = RP-2.

observed after only one week's standing on the bench. The change which does not occur in low actinic glassware was noted from the formation of a progressive purple tint in the colorless solution. Subsequent attempts to monitor this product(s) by HPLC proved unsuccessful. This product(s) could be an important intermediate in the decomposition mechanisms of aminocarb and warrants further investigation.

Phosphate buffer solution of pH 6.9 is used in the mobile phase to enhance the partitioning of the aminocarb phenol. Use of deionised-distilled water of pH 5.0 causes the phenol to tail very badly on the column compared to the sharply resolved peak in Figure 2. This property did not markedly manifest itself with the two other phenolic derivatives (1 and 2). The only notable difference between 2 and 5 is the secondary amino group versus the tertiary group and this leads to a tentative explanation that the buffer suppresses ionization of the solute thereby contributing to a greater affinity for the bonded phase.

As expected, the UV absorption and fluorescence detectors both produced linear responses. Detection limits using a signal to noise ratio of 2:1 were 0.5 ug/L and 0.05 ug/L respectively, assuming that a 1.0 L sample was analysed. Recoveries ranging from 76% were obtained using XAD-4 resin columns to extract aminocarb from fortified lake water (Table 1).

TABLE 1

Recovery of aminocarb from fortified lake water

Sample Volume (L)	Concentration (ug/L)	Mean Recovery[a] (%)	R.S.D. (%)
1.0	1.0	84	5.7
4.0	0.25	76	7.9
4.0	0.05	83	4.7

[a] Six determinations each, duplicate injections.

The procedure was applied successfully in another study involving the collection of rain water and was found quite suitable for the longer composite sampling periods. The persistence of this carbamate in river water is low according to EICHELBERGER et al. (1971) where only 10% was accounted for after two weeks. MALLET (1979) reported that aminocarb was stabilized for at least two weeks following extraction on an XAD-2 resin column. Investigations by the authors found the correlation to apply similarly for the XAD-4 resin where only 4% loss was detected after the second week, 13% after four weeks and 21% after six weeks standing on the laboratory bench.

In general results correlated well for rain water samples analysed by both HPLC and GLC as shown in Table 2. Noteable discrepancies are although apparent in the fourth and last sample and are probably due to solvent evaporation of the GLC aliquots. Analysis had been delayed because of the long shipping distance to the other laboratory responsible for the analysis. This trend follows for all samples and furthermore the positive bias may also result from the standard purities and/or some GLC on-column decomposition.

TABLE 2

Comparison of rain water data obtained by HPLC and GLC

Sample Size (L)	HPLC[a] (ug/L)	GLC[b] (ug/L)
3.85	0.15	0.17
3.56	0.16	0.19
4.22	0.27	0.32
2.33	0.30	0.39
2.28	0.33	0.36
4.05	5.1	6.8

[a] Fluorescence detector

[b] Thermionic nitrogen - phosphorous detector

Five other carbamates were analysed on the RP-8 column, namely: propoxur, carbofuran, carbaryl, methiocarb and mexacarbate, Under the 40:60 mobile phase system the first two were not resolved from aminocarb but this did not represent a serious problem because they are both non-fluorescent. AFGHAN (1979) observed similar results on a Chromegabond MC18 column and other carbamates apparently did not interfere in the analysis.

This study has shown that HPLC used in conjunction with UV absorption and fluorescence detection is a reliable and accurate technique to determine aminocarb. With modifications and further evaluation it could easily be improved and expanded to screen for the presence of decomposition products or metabolites in various substrates. Amberlite(R) XAD-4 resin was demonstrated to be particularly useful as a pre-concentration technique for large samples especially those collected over prolonged periods when degradation would otherwise take place.

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