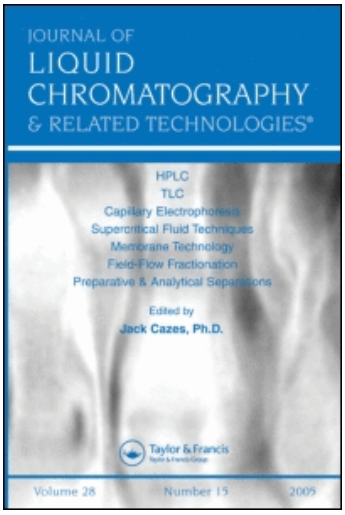

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LIQUID CHROMATOGRAPHIC ASSAY OF AMINOCARB AND FENITROTHION IN PESTICIDE FORMULATIONS

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ABSTRACT

A simple, rapid and robust liquid chromatographic method for the analysis of oil-based and emulsion spray-mixes containing aminocarb and fenitrothion insecticides is reported. The extracts of the spray-mixes, after necessary method optimization, were analysed using an HP RP-C8 column (200 x 4.6 mm ODS, 5 μm) with UV detection and methanol/water (85/15 v%) as the mobile phase. The linear concentration ranges for aminocarb and fenitrothion were 0.05 to 5.0 μg and 0.10 to 4.2 μg, respectively. Limit of detection and limit of quantification were 0.04 and 0.08 μg (aminocarb) and 0.05 and 0.10 μg (fenitrothion), respectively, in 20-μL injection volume. Analysis of different oil-based and emulsion spray-mixes of the two insecticides gave reproducible values with low CV, and agreement between the expected and measured values was good. The method could be modified and adapted for the trace analysis of the analytes from forestry matrices.

INTRODUCTION

Fenitrothion [O, O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate] and aminocarb [4-(dimethylamino)-3-methylphenyl methylcarbamate] are insecticides of economic significance used extensively in Canadian forestry since the early 1970's to control the defoliating lepidopteran insect pests.[1] Both insecticides are effective, economical, easy to handle, and are applied aerially as aqueous emulsions or as oil solutions. Several gas chromatographic (GC), high pressure liquid chromatographic (HPLC), thin layer chromatographic (TLC) and spectrophotometric methods for the determination of aminocarb and fenitrothion residues found in various matrices, and the contents of active ingredient (AI) present in their formulations, have been reported.[2-8] Among them, the traditional GC method is very sensitive; nevertheless, it is problematic because of the heat-labile character of aminocarb,[9-11] and instability and isomerization potential of fenitrothion above 200°C, especially while using metal columns in GC.[7] Time consuming derivatizations, accompanied by solvent partitions and column cleanup, are often required to overcome these problems.

Quality assurance and quality control of the spray-mixes used in forestry have become very high priorities during spray operations. Usually, the spray-mixes used in forestry spraying contain a single AI mixed with solvents, stickers, surfactants and other adjuvants to enhance target deposition and coverage. The company methods are usually suitable for the pure AI and for the commercial formulation concentrates, rather than for the spray-mixes containing a variety of additives and solvents added to the concentrates prior to the spray application, and these additives are normally prone to interfere either in the GC or HPLC analysis. Furthermore, the spray season in forestry is usually very short and a good number of emulsion and oil-based spray-mixes have to be analysed on time and on short notice. Therefore, robust, rapid and reliable methods to analyse and quantify the AI in the spray-mixes are required. Herein, we report a simple and sensitive reversed-phase HPLC method with UV detection to analyse the AI components present in the spray-mixes containing either aminocarb or fenitrothion insecticide.

MATERIALS AND METHODS

Analytical Standards

Analytical grade aminocarb (> 98% purity) and fenitrothion (> 97% purity) were supplied by Chemagro Chemical Co., Toronto, ON, and Sumitomo Chemical Co., Osaka, Japan, respectively. Both insecticides (100.0 mg) were dissolved separately in ethyl acetate and methanol and diluted to 100.0 mL in volumetric

flasks to give 1000 μg/mL solutions. The ethyl acetate stock solution of either insecticide was stable for the entire study period (> 7 weeks), whereas the methanolic solution of aminocarb developed a light brown colour after prolonged storage. To circumvent the problem, fresh solutions of the insecticides in methanol were prepared every two weeks. A 30.0-mL aliquot of each stock solution was diluted to a 100-mL volume in a volumetric flask with the same solvent to give a 300 μg/mL stock standard solution of each insecticide. Solutions of different concentrations were prepared by diluting this stock standard solution. All standards were kept in tightly sealed volumetric flasks in darkness at 1°C and filtered prior to injection into the HPLC system. Twenty-μL volumes of the standard solution of each insecticide at different concentrations were injected, in triplicate, into the HPLC and the detector response was measured for each in terms of peak area. Calibration curves were prepared by plotting the average peak area (y-axis) against the mass of the analytes (x-axis). Quantitation was done by comparing the peak area of the test material to that of the standard in the calibration curve and computing the concentration therefrom.

Solvents

Spectroquality solvents of ethyl acetate, acetonitrile, and methanol (J.T. Baker) were obtained from Canlab, Toronto, ON. Pure water, used throughout this study, was prepared by passing distilled water through a Milli-Q® water purification system (Millipore Co., Bedford, MA). Solvents were filtered using 0.20-μm pore size Nylaflo filters (Gelman Sciences Inc., Rexdale, ON). The mobile phases (acetonitrile/water and methanol/water) were filtered (0.20-μm pore size) and degassed prior to use.

Spray-Mixes

The selected spray-mixes of aminocarb and fenitrothion used in the present study are given in Table 1 along with the approximate AI concentrations, the generic names of the additives known to be present in them, and the suppliers. The composition and exact chemical nature of the additives in spray-mixes are proprietary information of the manufacturers.

Instrumentation

All chromatography was carried out using a Hewlett-Packard (HP) (Palo Alto, CA), model 1084B HPLC, equipped with a UV variable wavelength (190 to 600nm) detector, dual solvent systems and associated gradient pumps, an HP 79849

Table 1

Composition of the Spray-Mixes

Spray-Mix	Composition of Spray-Mix	Expected AI Concentration (g/mL)	Dosage (g AI/ha)	Application Rate (L/ha)
A-O[a]	Metacil 180 FO[b] + I.D. 585[c]	0.048	70	1.46
A-EC[d]	Metacil 180 F.E[e] + I.D. 585 + Atlox[f] + Water	0.048	70	1.46
F-O[g]	Sumithion-O[h] + I.D. 585	0.110	210	1.50
F-EC[i]	Sumithion-EC[j] + Triton X-100[k] + Water	0.110	210	1.50

[a] Aminocarb-oil
[b] Aminocarb, oil-based formulation (Chemagro Chem. Co., Toronto, ON)
[c] Petroleum distillate consisting of aromatics (Shell Canada Ltd., Toronto, ON)
[d] Aminocarb-emulsion
[e] Aminocarb,emulsion formulation
[f] Emulsifier (Atlas Chem. Industries, Brantford, ON)
[g] Fenitrothion-oil
[h] Fenitrothion, oil-based formulation (Sumitomo Chem. Co., Osaka, Japan)
[i] Fenitrothion, emulsion
[j] Fenitrothion, emulsion formulation
[k] Emulsifier, sticker and spreader (Rohm and Haas Canada Inc., West Hill, ON)

auto-sampler and variable volume Rheodyne® injector. All instrument control and data collection were done using a microprocessor controlled electronic integrator linked to an LC terminal (HP 79850 B). A full description of the instrument was given in an earlier publication.[12] The operating parameters were as follows:

Columns: (1) Zorbax C-18, 250 x 4.6 mm ODS, 10-μm diam.
(2) HP C-8, 200 x 4.6 mm ODS, 5-μm diam.
(3) Whatman Partisil C-18, 250 x 4.6 mm ODS, 10-μm diam.
(4) Regis C-18, 150 x 4.6 mm ODS, 5-μm diam.

Column pressure: 1.6×10^3 to 11.6×10^3 kPa

Mobile phase: Acetonitrile/water, methanol/water (5, 10 or 15 v% of water, isocratic for the first 8 min followed by 100% acetonitrile or methanol for the next 10 min to flush out the late eluters).

Flow rate: 0.5 and 1.0 mL/min

Oven temperature: 30 and 50°C

Injection volume: 20 μL

Concentration: Aminocarb standard, 2.5 to 250 μg/mL (0.05 to 5.0 μg per injection);
Fenitrothion standard, 5.0 to 210 μg/mL (0.10 to 4.2 μg per injection)

Attenuation: 5.12×10^{-2} AU/cm

Chart speed: 0.5 cm/min

Wavelength: Aminocarb, 248:430 nm (sample:reference);
Fenitrothion, 270:430 nm (sample:reference)

Run time: 18 min

Sample Preparation

The spray-mixes of aminocarb and fenitrothion formulations used in the aerial spray programs were shaken well on a wrist-action shaker for 0.5 h. Aliquots (0.3-0.5 mL) were weighed separately into 50-mL volumetric flasks and made up with methanol. Each solution was shaken for 0.5 h for complete analyte extraction, allowed to settle, and an aliquot was filtered (Nylaflo filter, 0.20-μm pore size) to remove particulates. The filtrate was transferred quantitatively to a graduated, stoppered centrifuge tube and the volume was adjusted either by concentration under

N_2 (Meyer N-Evap®) or by dilution with methanol, so that the concentration of the desired analyte in the test sample was within the concentration range of the calibration curve prepared using the standard. A 20-μL volume of the methanolic extract of each spray-mix was injected several times (n = 6) into the HPLC. The average peak area was calculated and the concentration of each analyte was computed from the respective calibration curves.

Method Optimization

A systematic approach to methods development task has been to research and find reliable assay conditions to separate and quantify aminocarb and fenitrothion from their respective spray-mixes. During the initial stages, column selection and the choice of mobile phase with its proper composition were done by trial and error, in order to get good resolution of the peaks of the target analytes. The resolution pattern of each insecticide was studied by using, successively, the four columns listed in the instrumentation section and injecting into each of them, several times, 20-μL volumes of the standards. Different ratios of acetonitrile/water and methanol/water were tried as mobile phases, and their resolution patterns were examined. The same procedure was repeated using sample solutions prepared from the individual spray-mixes.

Consistently efficient, good and reproducible resolution of the peaks of aminocarb and fenitrothion from the respective spray-mixes was possible only by using the HP C-8, 200 x 4.6 mm ODS, 5-μm column with methanol/water (85/15 v%) as the mobile phase. The use of C-8 column gave good resolution of the analyte peak and separation was relatively fast (run time, 18 min) with good efficiency (sharp and narrow peak), thus permitting a high output of sample analyses. As an added benefit, column deterioration after prolonged use was found to be minimal and the column life was excellent. Because the column packing was efficient, no guard column was used (there was some concern that such a pre-column would reduce the efficiency of the analytical column). The C-18 columns used in this study were more hydrophobic; therefore, their separation, selectivity and resolution potentials were poor and retention times (RTs) were longer, thus increasing the analysis time. Also, columns with 10-μm particle size had higher run times compared to 5-μm particle size columns. The peak resolution and efficiency were poor with the shorter Regis C-18 150 x 4.6 mm column, and plugging of the frits occurred occasionally which negated the separations. Although Zorbax and Whatman columns were similar in their size and bonded-phase packing, poor peak resolution and variability in RTs were observed during the preliminary assay studies, which resulted in abandoning further trials with them.

In optimizing the mobile phase selection, 85% methanol and 15% water mixture was the primary choice because of good resolution of the analytes in the test samples, producing distinct, sharp and narrow peaks with peak asymmetry factors around 0.85 to 1.15. However, trial studies showed that acetonitrile/water mixtures could also serve as an excellent substitute for methanol/water as mobile phase because of their lower viscosity (reduced back pressure) and higher solvent strength. Nevertheless, the relatively higher cost of acetonitrile, and its reported toxicity and related disposal problems, precluded its use in the present study. Although methanol/water did serve as an excellent mobile phase to separate the analyte peak, some band overlapping of impurity peaks was observed, especially while analysing the oil-based spray-mixes. Since there was no interest in those impurity peaks, no attempts were made to separate the bands.

Band spacing and baseline noise (*ca* 0.0001 AU) were found to be good at the column temperature of 30°C. However, at 50°C the RTs of peaks were decreased, reducing their band spacings, which in turn negatively affected the peak resolutions, especially in the oil-based spray-mixes; also, there was enhanced background noise. Similarly, using the mobile phase flow rate of 0.5 mL/min increased the RTs, which increased the analysis time. On the other hand, a flow rate of 1.0 mL/min not only reduced the analysis time but also enhanced the separation efficiency. Finally, to avoid the back pressure build-up with time, especially while using methanol/water as mobile phase, and to improve system reliability, an optimum column pressure of 3.5×10^3 kPa was chosen and used during the study. Prolonged use of the HP RP C-8, 5-μm column occasionally produced detectable impurities (adjuvants in spray-mixes, additives from the column packing and their degradation products etc.) as late eluters, and this effect was reduced by washing the column daily with methanol.

RESULTS AND DISCUSSION

Linearity of UV Detectors

The linearity of the UV detector to aminocarb and fenitrothion was checked by injecting 20-μL aliquots of each analyte standard in triplicate. Detector response (average peak area) was plotted against the concentration of each insecticide standard. The calibration curve was linear in the concentration range of 0.05 to 5.00μg for aminocarb and 0.10 to 4.20 μg for fenitrothion, in 20-μL injection volume. The curve passed through the origin with "r" values of 0.998 for aminocarb and 0.990 for fenitrothion.

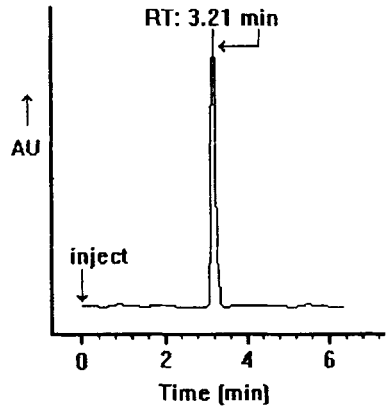

Figure 1. Liquid chromatogram of aminocarb after injecting 20 µL of a 50 µg/mL standard.

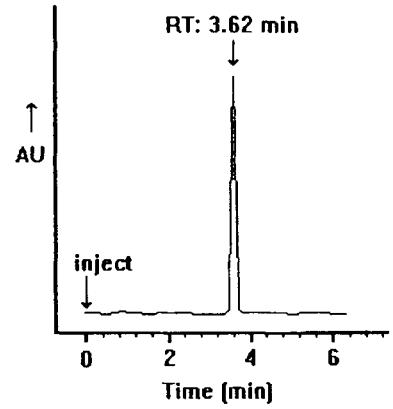

Figure 2. Liquid chromatogram of fenitrothion after injecting 20 µL of a 50 µg/mL standard.

The reproducibility in the peak area measurements at the above concentration ranges of aminocarb and fenitrothion were 96 and 92%, respectively. The average percent standard deviation (SD) observed in the peak area measurements for all the concentrations of the standards injected was 7 for aminocarb and 11 for fenitrothion.

Limit of Detection and Limit of Quantification

In the analysis of pesticide formulations and spray-mixes, the emphasis is placed on the content of the analyte in the test sample, as opposed to trace analysis where the emphasis is primarily to find the chemical in the matrix at residue levels (μg to ng/g). In the former case, the AI is usually a major component, the sample size is large and higher quantities of the prepared sample can be injected into the liquid chromatograph if the analyte has a weak chromophore. It is a macromethod, and of necessity, accuracy and precision are of fundamental importance. In such situations, the concept of limit of detection (LOD) and limit of quantification (LOQ) may not be applicable. However, from the observed linearity over the concentration ranges studied (0.05 to 5.00 μg in 20 μL for aminocarb and 0.10 to 4.20 μg in 20 μL for fenitrothion) and from the baseline noise of the chromatograms of the two insecticide standards, the LOQ values for aminocarb and fenitrothion were conservatively established as 0.08 and 0.10 μg, respectively. LOD values were 2 orders lower than the LOQs, i.e., 0.04 μg for aminocarb and 0.05 μg for fenitrothion (in 20 μL).

HPLC Chromatograms of the Standards

Typical chromatograms of aminocarb and fenitrothion standards, obtained by injecting 1.0 μg in 20 μL onto the HP C-8, 200 x 4.6 mm ODS, 5-μm column, are given in Figures 1 and 2. Each analyte peak was well resolved, narrow and symmetrical, showing that the selection of column, mobile phase and maximum absorption wavelength (248 nm for aminocarb and 270 nm for fenitrothion) were appropriate for the two insecticides. The RTs of aminocarb and fenitrothion were 3.21 and 3.62 min, respectively.

HPLC Chromatograms of the Spray-Mixes

The commercial formulations of aminocarb (Matacil) and fenitrothion (Sumithion) contained aromatic hydrocarbons, especially polyalkylated benzenes, as solvents.[7,8,13] In addition, the spray-mixes contained I.D. 585, Atlox and Triton as additives (Table 1), all of which are aromatic in origin. These materials also absorb in the UV region[13] and were eluted along with the analytes of interest. Fortunately, all the aromatics present in the spray-mixes emerged from the column either before or after the components of interest, as seen in Figures 3 to 6. The peaks corresponding to each analyte were distinct, fairly symmetrical, free from interference and resolution was quite satisfactory.

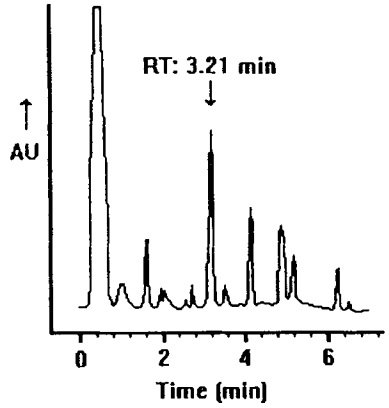

Figure 3. Liquid chromatogram of A-O spray-mix of aminocarb after 20-μL injection (see Table 1 for spray-mix details).

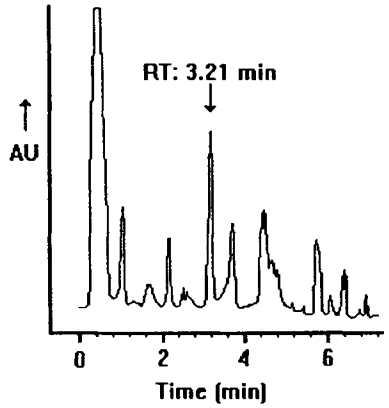

Figure 4. Liquid chromatogram of A-EC spray-mix of aminocarb after 20-μL injection (see Table 1 for spray-mix details).

Aminocarb and Fenitrothion Contents in the Spray-Mixes

Replicate analysis (n = 6) of the aminocarb (Matacil) spray-mix, A-O, showed that it contained, on average, 4.64% of the insecticide (range 4.51 to 4.86%) or 97% of the expected value (Table 1). The corresponding value for the A-EC spray-mix

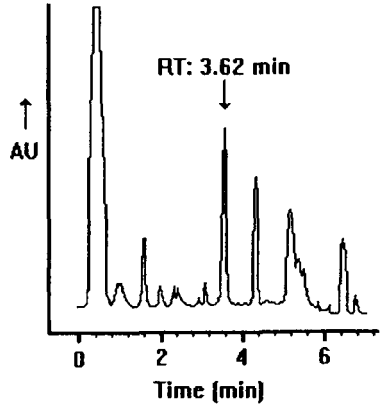

Figure 5. Liquid chromatogram of F-O spray-mix of fenitrothion after 20-µ1L injection (see Table 1 for spray-mix details).

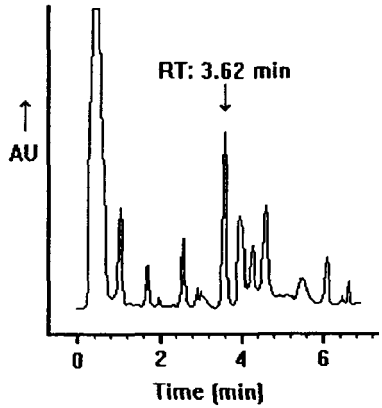

Figure 6. Liquid chromatogram of F-EC spray-mix of fenitrothion after 20-µL injection (see Table 1 for spray-mix details).

was 4.53% (range 4.48 to 4.71%) or 94% of the expected value. The F-O spray-mix of fenitrothion contained 11.2% AI (range 10.9 to 11.8%), corresponding to 102% of the expected value. On the other hand, the value for F-EC spray-mix was only 9.41% AI (range 8.30 to 9.91%) or 86% of the expected value, indicating possible hydrolysis of the fenitrothion ester in the aqueous mixture.

To determine the precision of the method, each spray-mix was stored at 4°C in darkness and re-analysed each day for the following two days. The mean AI (%), ± SD and CV (%) for the spray-mixes were 4.61, 0.06 and 0.71 (A-O); 4.51, 0.07 and 0.63 (A-EC); 11.1, 0.08 and 0.92 (F-O); and 9.2, 0.11 and 1.12 (F-EC), respectively, indicating good precision.

The HPLC method reported in this paper was simple, sensitive and rugged, and the data on analyte contents showed good agreement between the expected and measured values. Based on the results of this study, it is apparent that the method is useful to analyse aminocarb and fenitrothion contents in forestry spray-mixes used in insect control programs. With necessary modifications, such as solvent extraction, partitioning, column cleanup and sensitivity optimization, the method could be extended to trace analysis of aminocarb and fenitrothion residues found in various forestry matrices following aerial spray applications.

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