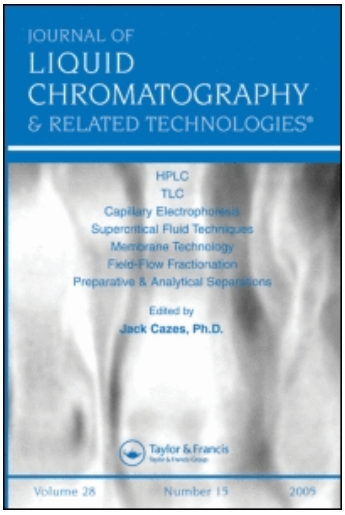

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ANALYSIS OF THE CARBAMATE INSECTICIDES ALDICARB AND CARBARYL IN FORMULATIONS UTILIZING A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SYSTEM WITH AN ON-LINE INFRARED DETECTOR

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ABSTRACT

An aldicarb (2-methyl-2-(methylthio)propanal, O-[(methylamino)-carbonyl]oxime) granular formulation with and without added carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) as an internal standard and a carbaryl (1-naphthalenyl methylcarbamate) wettable powder and liquid suspension formulations both with and without added methiocarb (3,5-dimethyl-4-(methylthio)phenyl methylcarbamate) as an internal standard were analyzed by utilizing a high-performance liquid chromatographic system operating in the normal phase with a Partisil column and mixtures of acetonitrile:dichloromethane:heptane as the mobile phase. An on-line infrared detector equipped with a flowcell was used. Analyses were conducted at ambient temperature. The commonly used calibration and quantitation techniques were also compared.

INTRODUCTION

The N-methylcarbamate compounds comprise a major class of insecticides. Although carbamates have been used for a long time in pest control operations, analytical methodology for their quantitation is still in the state-of-the-art relative to other classes of established pesticides. Gas chromatography (GC) generally has failed to qualify as a direct analytical tool due to the unfavorable chemical and physical properties of the carbamate insecticides

and frequently to the lack in their chemical structure of elements amenable for their detection by the generally used GC detectors. The available analytical methodologies, as well as the problems associated with them, have been reviewed by Dorough & Thorstenson (1), Magallona (2), and recently by Seiber (3).

The rapid development of high-performance liquid chromatography (HPLC) into a powerful analytical tool for trace analysis has led to a rapidly increasing number of applications to pesticide analysis in the last five years. HPLC has become a promising alternative for the compounds troublesome to analyze under GC conditions. The major drawbacks in HPLC analysis of pesticides today arise from a need for a detection system which is easily operated on the routine basis, trouble free and selective. The most commonly used detector in HPLC analytical systems is the ultraviolet (UV) detector. Moye (4), after reviewing the applications of HPLC in pesticide residue analysis, reported that in more than 85% of the cases, UV was used as the detection system; therefore except for special cases of relatively clean extracts, very extensive sample cleanup is required. Post-column hydrolysis and fluorogenic labeling has been applied by Krause (5) for the residue analysis of N-methylcarbamates.

Infrared (IR) spectroscopy has had a long history of use in pesticide analysis before the advent and wide use of GC, and later as a GC detector for on-the-fly GC effluents. The inherent low sensitivity of the conventional IR spectrophotometers and the constantly increasing emphasis on the development of more sensitive detection systems in pesticide residue analysis has diminished the use of IR. Fourier transform IR (FT-IR) interferometers as GC detectors have overcome the sensitivity deficiencies and have permitted the use of valuable IR spectral information for the positive identification of the sought analytes. However, the high cost of FT-IR instrumentation restricts its use for routine pesticide analysis. The utilization of an IR photometer on-line to the HPLC eluate via a flowcell for the analysis of pesticides was first applied for the formulation analysis of the pyrethroid insecticides

(6,7). The system was demonstrated to be very efficient in terms of selectivity, reproducibility and reliability for the analysis of emulsifiable and aerosol concentrates by direct injection on the HPLC after appropriate sample dilutions. The main problem was the relatively low sensitivity which can be either a curse or a blessing depending on one's objective and analytical problem. If it is desired to apply the system to residue analysis, the sensitivity problem can be resolved by increasing the injection volume and/or the amount of the extracted sample. Preliminary research in applications to residue analysis has shown that very concentrated solutions of a variety of plant extracts do not interfere with the analysis. A minimal cleanup is required only for the protection of the HPLC column.

The application of the HPLC-IR system for the analysis of carbamates is demonstrated below with the analysis of two commonly used carbamate insecticides, aldicarb and carbaryl. The commonly used calibration and quantitation techniques are also compared.

EXPERIMENTAL

Reagents and Solvents

Analytical grade (99.9%) aldicarb and carbaryl were obtained from Union Carbide Corporation. Analytical grade (99%) carbofuran was purchased from Chem Service and methiocarb (97.9%) was obtained from Mobay Chemical Corp. An aldicarb granular formulation and two carbaryl formulations, a wettable powder (80% wt/wt) and a liquid suspension (4 lb/gal) were donated by Dr. G. Carman (Department of Entomology, University of California, Riverside). All solvents were "distilled-in-glass" from Burdick & Jackson Laboratories, Inc. Solvents were filtered through 25-grade glass filter (Schleicher & Schuell) and degassed by shaking under reduced pressure just prior to use. A mixture of acetonitrile:dichloromethane:heptane was used as the mobile phase in the ratio of 15:30:55 (v/v/v) for carbaryl analysis and 20:40:40 (v/v/v) for aldicarb analysis. The appro-

priate mobile phase composition was controlled by a solvent programmer operated under isocratic conditions and constant flow rate of 1.5 mL/min.

Instrumentation

The HPLC system consisted of two Waters Associates Model 6000A pumps controlled by a Waters Associates Model 660 solvent programmer. A Rheodyne (Cotati, CA) 7125 valve injector equipped with a 20-μL loop was used. A Foxboro Wilks Miran-1A (El Monte, CA) variable wavelength IR filter-type photometer was used as the detection system; a 1.5-mm light path and 4.5-μL capacity BaF_2 flowcell was used. A 5 cm x 4.6 mm i.d. Whatman HC Pellosil guard column was used to protect the 25 cm x 4.6 mm i.d. Whatman 10-μm Partisil analytical column. Column fittings were of low dead-volume unions and 0.25 mm i.d. tubing was used to made the necessary connections.

Sample Preparation

Formulations were extracted according to the official AOAC methods (8) with minor modifications. A 1.2 g sample of aldicarb granular formulation was extracted by mechanical shaking with 100 mL of dichloromethane for 2 h (extract I) or Soxhlet-extracted at 5 cycles/h with 80 mL of dichloromethane for 3 h (extract II) and 4 h (extract III). The Soxhlet extracts were made up to 100 mL final volume with dichloromethane. A 10 mL fraction of each extract was filtered through 25-grade glass filter and was subjected either directly to HPLC analysis or after the addition of carbofuran internal standard to 7 mL of filtered extract and dilution to 10 mL by the addition of dichloromethane (diluted fraction); final carbofuran concentration of 3 mg/mL.

A 3.125 g sample of carbaryl wettable powder was extracted by mechanical shaking for 30 min in 50 mL of 10% acetonitrile in chloroform (v/v). The suspension was centrifuged for 5 min at 2000 rpm. The volume of the supernatant was recorded and a 10 mL fraction was filtered through 25-grade glass filter. One mL of the

filtrate was diluted to 25 mL by the addition of mobile phase and subjected directly to HPLC analysis or after the addition of methiocarb internal standard to give a final concentration of methiocarb of 1 mg/mL. A 0.5 mL (0.6 g) sample of the liquid suspension carbaryl formulation was extracted by mechanical shaking for 30 min with 100 mL of chloroform after the addition of 20 g of anhydrous Na_2SO_4. A fraction of the supernatant was filtered and directly analyzed by HPLC or after dilution of 7.5 mL to 10 mL with mobile phase with the simultaneous addition of methiocarb to give a final methiocarb concentration of 1 mg/mL.

The injection volume was kept constant at 20 μL by using the loop-filling technique. At least three replicate injections were made of each analyzed sample. All standard solutions for both aldicarb and carbaryl analyses were made in the corresponding mobile phases.

Calibration Methods and Quantitation

Both the external and internal standard calibration methods were used. Carbofuran was used as the internal standard at the final concentration of 3 mg/mL for the analysis of aldicarb, and methiocarb at 1 mg/mL for the analysis of carbaryl. Therefore, two calibration curves were made for each compound, one by plotting peak heights in cm *vs.* μg of the analyte, and the other by plotting the ratio, (peak height of the analyte)/(peak height of the internal standard), *vs.* μg of the analyte. The calculations were made on the basis of the corresponding calibration factors (slope of the calibration curves) expressed either as peak height in cm/μg of analyte (Method A), or the ratio of peak heights (analyte/internal standard) /μg of the analyte (Method B), or a one point calculation of the calibration factor (Method C). In the last method a standard curve was not constructed but the calculations were based on the response of a standard solution of the analyte of a certain concentration. This last standard solution was selected from among the ones used for the above-mentioned calibration curves and contained the appro-

priate standard at a concentration within ±1 µg/uL of the unknown sample.

RESULTS AND DISCUSSION

Carbamates with insecticidal activity are either aryl or oxime N-methylcarbamates. A common feature of the IR spectrum of all carbamates is the presence of the strong carbonyl absorption band in the area of 5.7 to 6.3 µm. Preliminary studies on the chromatographic behavior of the carbamate insecticides in normal phase liquid-solid chromatography (LSC) on a silica gel column showed that all compounds can be separated and eluted from the column with solvent combinations which are relatively transparent at the carbonyl absorption range.

The ultimate purpose of a trace analysis is the identification and quantitation of the sought analyte(s). In LC quantitative analysis, sources of error are: sampling, choice of the chromatographic conditions, detection, calibration of the method, and measurement (9). With the use of microvalve injectors, the sampling error can be minimized. The choice of the proper chromatographic conditions for a specific separation is an important factor. A brief review of the literature shows that certain groups of compounds are preferentially analyzed by one specific chromatographic mode. Furthermore, the selection of the proper mobile phase composition is critical to obtain the desired separation since undetected or unsuspected overlapping peaks can create erroneous quantitative results. Snyder & Kirkland (9) list the desired characteristics of an ideal LC detector. Measurements can be made either by measuring peak areas or peak heights depending on the conditions. It is generally believed that calibration methods with internal standards can increase the precision of the analysis. This is true if a pretreatment or derivatization of the sample is involved. However, too much weight is given to the use of the internal standard independent of the other analytical conditions. Publications appear which present methodologies which utilize internal standards without

justifying its presence; additional effort is required in finding a chemically and chromatographically appropriate and readily available compound to serve as the internal standard for a specific separation. It is also possible to increase the precision error of an analysis due to the possible low resolution of the internal standard from the compound(s) of interest, the interactions of the internal standard with coextractives or primary metabolites of the pesticide, and the differential stability in solution of the internal standard and the sought analyte even under refrigerated conditions as in the case with the N-methylcarbamates. Furthermore, one can actually increase the measurement error since one has to measure two peaks instead of one. Therefore, the different calibration and calculation methods commonly used in quantitative LC were compared here in terms of accuracy and reproducibility.

Aldicarb. A sample chromatogram of an aldicarb formulation extract is shown in Figure 1 and the results of the analysis are given in Tables 1 and 2. The analysis was conducted three times, spaced every other day. The measurements were made according to the three methods as explained in the experimental section. The calibration factors based on standard curves for each date of analysis were: 0.121, 0.124 and 0.133 cm/μg of aldicarb without an internal standard and 0.021, 0.021 and 0.022/μg of aldicarb in the presence of the internal standard. There is a small but consistent increase of the slope of the standard curve in the first set of values apparently due to the concentration of the standard solutions because of the high volatility of the solvent. This increase is not prominent in the second case since the presence of the internal standard compensates for the small changes in the aldicarb concentrations. In Table 1 are shown the results of the analysis of the undiluted aldicarb extract (without internal standard). The calculations were made according to Method A and C. The mean values considering the three dates as replicates for the three extracts are: 12.5±0.3, 12.3±0.2 and 12.5±0.3 from Method A and 12.6±0.6,

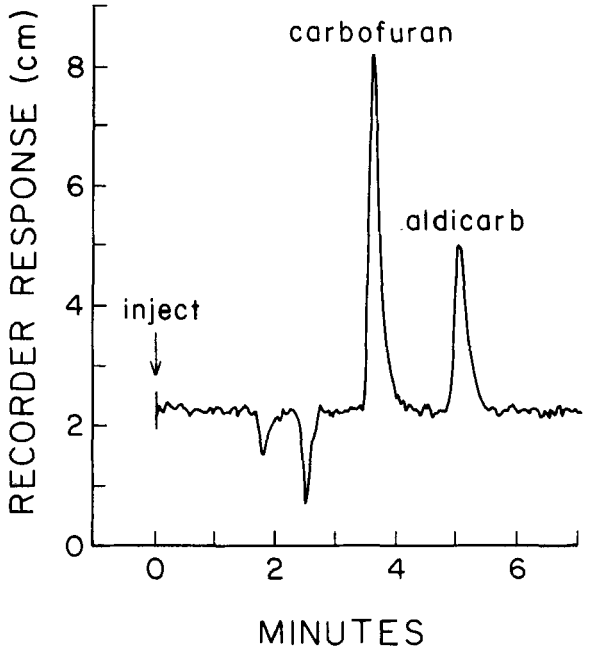

FIGURE 1

IR liquid chromatogram obtained for a 20-μL injection of an aldicarb granular formulation extract containing 3 mg/mL carbofuran as the internal standard. The IR detector was operated at 5.75 μm and 0.1 AUFS with a 1.5-mm path length, 4.5-μL capacity BaF_2 flowcell. The mobile phase consisted of acetonitrile:dichloromethane:heptane (20:40:40). Flow rate was 1.5 mL/min.

12.4±0.5 and 12.6±0.6 from Method C. Although the mean values obtained by either method of calibration and from all three extracts are not statistically significant at the 1% level, Method A appears to be more reliable (lower variance) for day to day analysis than Method C which showed a variance per date more than twice greater than from Method A for these three extracts analyzed.

In Table 2 are shown the results of the analysis of the diluted aldicarb extracts with the internal standard. Method C showed the smallest variance per date and Method A the highest for extract I. The order was reversed in extract III whereas in extract II Method

TABLE 1

Results, Expressed as % by Weight, of the Analysis of Aldicarb Extracts from a Granular Formulation[a/]

	Undiluted fraction without internal standard					
	Extract I		Extract II		Extract III	
Date	A	C	A	C	A	C
1	12.4	12.5	12.4	12.5	12.4	12.5
2	12.8	13.2	12.4	12.9	12.8	13.2
3	12.2	12.0	12.0	11.9	12.2	12.0
Mean	12.5	12.6	12.3	12.4	12.5	12.6
Std. Dev.	0.3	0.6	0.2	0.5	0.3	0.6
Variance	0.09	0.34	0.05	0.24	0.09	0.34

Grand Mean: 12.5±0.4

[a/] IR detector operated at 5.75 μm and 0.1 AUFS.

TABLE 2

Results, Expressed as % by Weight, of the Analysis of Aldicarb Extracts from a Granular Formulation[a/]

	Diluted fraction with carbofuran as the internal standard								
	Extract I			Extract II			Extract III		
Date	A	B	C	A	B	C	A	B	C
1	12.6	12.7	12.5	12.8	12.9	13.7	12.8	13.6	12.4
2	13.0	13.1	12.9	12.2	13.4	12.6	12.6	13.4	13.3
3	13.0	13.1	12.8	12.7	13.4	12.6	12.1	12.7	11.9
Mean	12.8	13.0	12.7	12.6	13.5	12.9	12.5	13.2	12.9
Std. Dev.	0.2	0.2	0.2	0.4	0.3	0.6	0.4	0.5	0.9
Variance	0.06	0.05	0.04	0.13	0.08	0.40	0.15	0.23	0.72
Grand Mean:	12.9±0.5								

[a/] IR detector operated at 5.75 μm and 0.1 AUFS. Each value is the mean of three injections.

B showed the smallest variance and Method C the highest. Applying the Bartlett's test of the homogeneity of variance, it was found that the pooled variance of all three methods over extractions for both diluted and undiluted samples were not statistically significant. Considering the pooled variance per date for each method separately over all extractions, there were not statistically significant differences at the 1% level for both diluted and undiluted extracts. Duncan's multiple range test of dates for each method showed dates not significantly different for Method A but they were different for Methods B and C. However, averages were taken over the three extracts.

Carbaryl. Two formulations of carbaryl were analyzed, a wettable powder and an aqueous suspension. Sample chromatograms of the analysis of both formulations are shown in Figure 2, and results of the analysis in Table 3. The analysis was repeated the next day. The

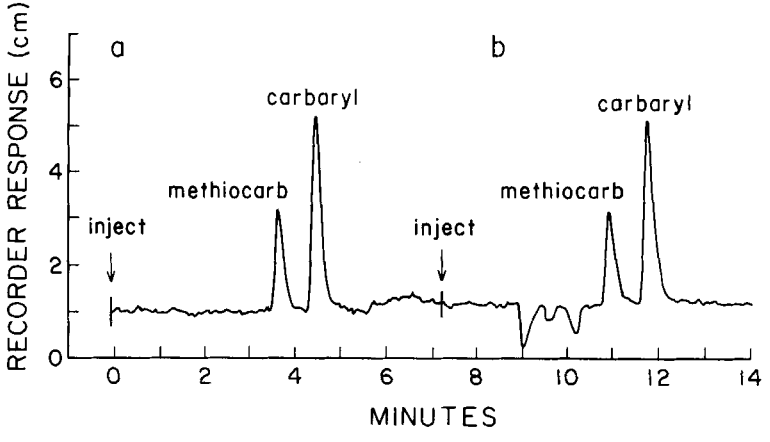

FIGURE 2

IR liquid chromatograms obtained for 20-μL injections of carbaryl formulation extracts: (a) wettable powder and (b) liquid suspension; each containing 1 mg/mL methiocarb as the internal standard. The IR detector was operated at 5.75 μm and 0.1 AUFS with a 1.5-mm path length, 4.5-μL capacity BaF_2 flowcell. The mobile phase consisted of acetonitrile:dichloromethane:heptane (15:30:55). Flow rate was 1.5 mL/min.

TABLE 3

Results, Expressed as % by Weight, of the Analysis of Carbaryl Extracted from a Wettable Powder and a Liquid Suspension Formulation[a/]

	With methiocarb as an internal standard					
	Wettable Powder			Liquid Suspension		
Date	A	B	C	A	B	C
1	77.5	77.6	78.9	43.3	43.3	45.0
2	77.5	77.6	78.9	43.0	41.6	42.6
Mean	77.5	77.6	78.9	43.2±0.2	42.4±1.3	43.8±1.7

[a/] IR detector operated at 5.75 μm and 0.1 AUFS.

calibration factors were: 0.102 and 0.111 cm/μg of carbaryl for each date of analysis and 0.05/μg carbaryl for both dates in the case where the internal standard was included. Again, the small increase of the slope in the first case is probably due to the concentration of the standard solutions whereas this change is compensated for by the presence of the internal standard. No statistical analysis was made for this set of results due to the small number of values, except for calculations of mean values and standard deviations per date for each method of measurement. The relatively high standard deviations of Methods B and C over dates in the case of the analysis of carbaryl liquid suspension is believed to be due to the difference in stability of carbaryl and methiocarb in the extraction solvent.

CONCLUSIONS

An HPLC system operated in the normal phase with a silica gel column and an on-line infrared detector has been demonstrated to be an easily operated, trouble-free system and has been successfully applied for the analysis of aldicarb and carbaryl in formulations. No interference from the formulation coextractives was present. The selected mobile phase combinations are relatively transparent in the 5.7 to 5.75 μm region of the infrared spectrum where these carbamates show their strongest absorption band and still have the proper strength and selectivity to separate and elute the analyzed carbamates in less than 6 min. Any calibration method can be used for the quantitative measurements since no statistically significant differences were found in the results obtained by either method. Therefore the selection has to be made on the basis of simplicity and the number of pitfalls associated with each method depending on the analytical problem.

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