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FLUORIGENIC LABELLING OF CARBAMATES WITH DANSYL CHLORIDE

IV. *IN SITU* QUANTITATION OF N-METHYL CARBAMATE INSECTICIDES ON THIN-LAYER CHROMATOGRAMS

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SUMMARY

The quantitative analysis of a number of N-methyl carbamate insecticides by thin-layer chromatography and *in situ* fluorometry of their dansyl derivatives is investigated. Statistical analysis indicates a relative standard deviation of 4.5 % for concentrations ranging from 5 to 500 ng per spot of carbamate. Calibration curves (peak area *versus* concentration) are linear up to 300–400 ng per spot and generally exhibit an increase in slope with time, due to an increase in fluorescence of the spots.

Analyses are performed on spiked water samples. Mesurol and Landrin are simultaneously extracted and reacted with dansyl chloride, after which the resulting dansyl-phenol derivatives are analysed quantitatively following separation on Silica Gel G–silver oxide layers with benzene–acetone (98:2). Baygon, Mesurol and Landrin are simultaneously extracted from water samples and determined as total N-methyl carbamate by measuring the fluorescence of the dansyl-methylamine derivatives. Recoveries from the samples extended from 90–106 % at concentrations in the low p.p.b.* range.

The method offers improved specificity over existing techniques because two derivatives are formed with each carbamate, both of which can yield quantitative results.

INTRODUCTION

The quantitative analysis of carbamate insecticides has been the subject of much research in recent years. These compounds are not easily analysed by gas chromatography (GC) because of their thermal instability, and, therefore, often complicated derivatization of carbamates is needed for GC analysis. Progress in this area has been reviewed by WILLIAMS[1]. These procedures, however, are not entirely satisfactory and often suffer from reagent or sample interferences which greatly decrease the sensitivity of the methods.

Thin-layer chromatography (TLC) has often been used for the analysis of

* Throughout this article the American (10^9) billion is meant.

carbamate insecticides, although until recently only in a qualitative or semi-quantitative capacity. These methods usually involve chromogenic spray reagents for the formation of coloured derivatives of carbamates directly on thin-layer chromatograms. The combination of TLC and fluorescence offers certain advantages over these techniques. Fluorescence is often 10–100 times more sensitive than colorimetric procedures and it is more applicable to quantitative analysis. The determination of a naturally fluorescent carbamate, Sevin (carbaryl, 1-naphthyl-N-methyl carbamate), and its breakdown product α-naphthol, by TLC and *in situ* fluorometry[2] proved the quantitative potential of such a method. This technique has been expanded to include the analysis of non-fluorescent pesticides[3,4]. In these papers fluorigenic spray reagents were developed for the analysis of a number of organophosphate and carbamate insecticides. Although these spray techniques are rapid and simple, they lack specificity and the sensitivity is only about ten-fold better than for the chromogenic spray procedures. An alternative to these spray methods is fluorigenic labelling, which involves the formation of fluorescent derivatives that are separated by TLC and quantitatively analysed by *in situ* fluorometry. This technique has the advantage of not requiring a spray reaction for the formation of the fluorescent spots. Thus, background irregularities due to uneven spraying are avoided.

Preliminary work on the analysis of carbamate insecticides by this fluorigenic labelling technique has been carried out by FREI AND LAWRENCE[5,6], who used dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride) as the labelling reagent. This compound reacts with both the amine and phenol hydrolysis products of carbamates, resulting in two derivatives suitable for the quantitation of carbamate residues in the low nanogram range. The reaction procedure has since been investigated[7] and results indicate that complete formation of the dansyl derivatives of the carbamates studied takes place in less than 1 h. The fluorescence behaviour of these derivatives was examined[8] in order to determine the stability of the derivatives with time and to investigate those conditions which might affect the quantitative results. The TLC of the dansyl derivatives of a number of carbamate insecticides has been investigated[9]. Several systems were developed for the one- and two-dimensional separation of these compounds. The actual application of the method to quantitative analysis of carbamates in water samples is the subject of the present study.

EXPERIMENTAL

Reagents

Analytical-grade dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride) obtained from Mann Research Laboratories (136 Liberty St., New York, N.Y. 10006, U.S.A.) was dissolved in redistilled reagent-grade acetone to form a 0.2% solution. The carbamates (listed in Table I) were recrystallized and checked by NMR and IR spectroscopy; stock solutions were prepared at a concentration of 1 mg/ml in redistilled reagent-grade methylene chloride. A 0.1 *M* solution of sodium carbonate was used for the hydrolysis of the carbamates and to buffer the coupling reaction. Reagent-grade solvents were used for the coupling reaction and chromatography. The spray solution consisted of 20% triethanolamine in isopropanol.

Reaction procedure

10 μl of a carbamate solution were placed in a 10-ml glass-stoppered test tube

TABLE I

CARBAMATE INSECTICIDES USED FOR THE PRESENT STUDY

Carbamates *Trade name*	*Chemical name*
Sevin	1-naphthyl-N-methyl carbamate
Mesurol	4-methylthio-3,5-xylyl-N-methyl carbamate
Baygon	2-isopropoxyphenyl-N-methyl carbamate
Landrin	3,4,5-trimethylphenyl-N-methyl carbamate
Matacil	4-dimethylamino-*m*-tolyl-N-methyl carbamate
Zectran	4-dimethylamino-3,5-xylyl-N-methyl carbamate
Bux	*m*-(1-ethylpropyl)phenyl-N-methyl carbamate and *m*-(1-methylbutyl)phenyl-N-methyl carbamate
Mobam	benzo(b)thien-4-yl-N-methyl carbamate
Carbofuran	2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methyl carbamate

with a Hamilton 10-μl syringe. The methylene chloride was evaporated by heating the test tube momentarily in a warm water bath. 1 ml of the sodium carbonate solution was added and the tube was heated for 30 min in the dark at 45°. Following this, three drops of the dansyl chloride solution were added and the test tube was shaken gently, heated for another 20 min in the dark at 45°, and then cooled to room temperature, after which 0.5 ml of *n*-hexane was added. The test tube was stoppered and shaken gently for 2 min. The phases were permitted to separate and 10 μl of the *n*-hexane layer were spotted on a TLC plate for separation of the derivatives.

Chromatography

Two types of TLC plates were used in this work. (1) Silica Gel G plates were prepared by applying a slurry consisting of 30 g of Silica Gel G (with calcium sulfate binder) (Macherey, Nagel and Co., Düren, G.F.R.) and 60 ml of distilled water, to 20 × 20 cm glass plates with a Desaga (Heidelberg, G.F.R.) applicator set at 250-μ thickness. (2) Silica Gel G–silver oxide plates were prepared from a slurry consisting of 30 g of Silica Gel G with the addition of 0.15 g of $AgNO_3$ in 20 ml of water, followed by 10 ml of 0.05 *N* NaOH and 30 ml of water. The plates were dried at room temperature and stored in the dark to prevent significant reduction of the silver oxide.

The chromatography of the dansyl derivatives was carried out in the dark by the ascending technique at room temperature. Benzene–acetone (98:2) was used as the eluting solvent for all separations. After reaching a solvent front of at least 13 cm, the plates were removed from the developing chamber, dried in a cool air stream and sprayed until visible moist with the triethanolamine in isopropanol solution to enhance and stabilize the fluorescence of the derivatives[8]. The isopropanol was then removed from the layer by placing the chromatoplates under a cool air stream for 2 min.

Instrumental analysis

The sprayed chromatoplates were evaluated with the Zeiss PMQ II chromatogram spectrophotometer in the fluorescence mode. The mercury lamp, in conjunction with the 365-nm filter, was used as the excitation UV light source. The emission

monochromator slit-width was set at 0.5 mm for all measurements. The emission wavelength was set to 530 nm for measurement of all derivatives, with the exception of the dansyl-phenol derivative of Mesurol, for which a setting of 525 nm was used. The speed of the scanning table was 15 cm/min. The readout device was a Honeywell Electronik 194 strip-chart recorder with the chart speed set at 40 cm/min. All peak areas were measured with a Gelman planimeter. Calibration curves were plotted as peak area *versus* concentration.

Extraction of water samples

Each spiked sample was prepared by adding 10 μl of a 0.1 mg/ml acetone solution of a carbamate to 500 ml of a water sample. The sample was then poured into a 1-l separatory funnel and extracted twice with 50-ml portions of redistilled methylene chloride. These were combined, dried over anhydrous sodium sulfate and transferred to a 250-ml round-bottomed flask for rotary vacuum evaporation at room temperature to a volume of about 2 ml. This was then transferred to a ground-glass-stoppered 10-ml test tube and evaporated until barely dry. Several drops of acetone were added to rinse down the sides and to dissolve the residue. This was then treated with sodium carbonate and dansyl chloride according to the reaction procedure. Aliquots (10 μl) of the *n*-hexane layer were spotted on a TLC plate in triplicate for each sample.

This procedure was carried out on samples containing only Mesurol, equal amounts of Mesurol and Landrin, and a combination of Mesurol, Landrin and Baygon. Each of these combinations was extracted from local tap water, lake water and sea water. The samples containing the three pesticides were measured for total carbamate only, which was determined by evaluating the amount of methylamine derivative formed.

RESULTS AND DISCUSSION

Fluorescence phenomena, calibration curves and detection limits

The fluorescence of the dansyl derivatives on Silica Gel G layers was found to be considerably enhanced by spraying the chromatoplate with some organic solvent[7]. The enhancement is due to the dissolution of the dansyl derivatives from their dry adsorbed state in which they are weakly fluorescent, into the organic liquid where they become highly fluorescent. Triethanolamine in isopropanol was very suitable for such a spray because it has a high boiling point and does not readily evaporate from the layer. The dansyl derivatives do not immediately dissolve into the triethanolamine after spraying. The dissolution process is slow, as can be seen in Fig. 1, which gives calibration curves for the dansyl-phenol derivative of Mesurol. An increase in fluorescence intensities of the spots is observed with time, causing the slope of the curve to increase slowly. This increase continues up to 42 h, after which time the fluorescence becomes stable. The over-all increase in fluorescence at this time is about 30%. A similar behaviour was noticed for most other carbamate derivatives except for the dansyl-phenol derivatives of Zectran and Matacil (for Zectran, see Fig. 2), both of which contain a dimethylamino group which seems responsible for their opposite behaviour. However, quantitative measurements are

possible for these derivatives, since the decrease in fluorescence is slow and the linearity of the curves is maintained.

The reproducibility of the spray technique in this procedure is not as critical a factor as it is in methods where the spray contains a reagent to produce fluorescence directly on the plate[3,4]. The triethanolamine is non-fluorescent, and consequently an excess in any one area of the plate will not result in an irregular background.

The calibration curves were found to pass through the origin and are linear up to 300–400 ng per spot for most carbamate derivatives.

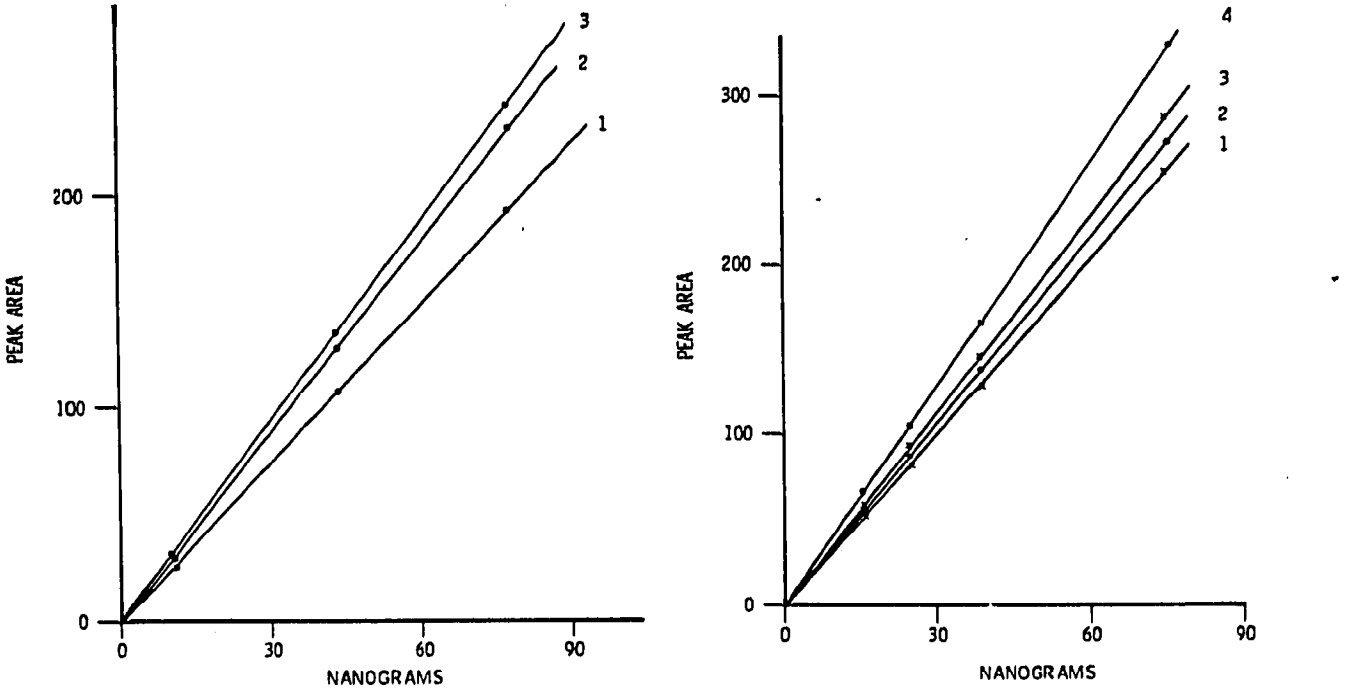

Fig. 1. Calibration curves peak area *vs.* concentration for the dansyl-phenol derivative of Mesurol as a function of time after spraying. Curve 1 = 30 min; curve 2 = 18 h; curve 3 = 42 h, 5 days.

Fig. 2. Calibration curves peak area *vs.* concentration for the dansyl-phenol derivative of Zectran as a function of time after spraying. Curve 4 = initial scan; curve 3 = 10 min; curve 2 = 20 min; curve 1 = 30 min.

Visual detection limits approach 5 ng or less per spot, while 1 ng can be detected instrumentally at a minimum of a 2:1 signal-to-background ratio. The above values vary slightly from carbamate to carbamate, depending on the R_F values of the derivatives. Higher R_F values will cause more diffusion and therefore poorer detection limits.

Reproducibility

The reproducibility of this method was determined by performing the labelling reaction simultaneously on nine separate carbamate samples of equal concentration and by spotting 10-μl aliquots of each for separation and analysis. Table II shows the results obtained over a concentration range of 5–500 ng per spot for the dansyl-phenol derivative of Mesurol. This study was performed in less detail on the phenol derivatives of Landrin and Sevin, with similar results. The relative standard deviation

TABLE II

REPRODUCIBILITY OF LABELLING TLC AND SUBSEQUENT FLUOROMETRY FOR THE DANSYL-PHENOL DERIVATIVE OF MESUROL

Concn. (ng per spot)	*Rel. standard deviation (%)*[a]				*Average (%)*
5	6.0	8.5	8.0	6.8	7.3
10	3.1	5.5	5.6	6.6	5.2
30	5.2	3.5	4.2	3.1	4.0
100	3.2	5.3	4.3	3.8	4.2
500	3.6	4.9	3.3	5.1	4.2

[a] Minimum of seven spots per plate.

remained fairly constant near 4.0–4.5 % above 10 ng per spot. At lower concentrations the error becomes appreciable since the limit of detection is being approached. Analysis in this region may be performed by using standard addition techniques[2,10] which bring the sample concentration into a more favourable range.

The stability of most of the derivatives, when covered and stored in the dark, is good even after three weeks. There was no change in excitation or emission maxima over this time, with the exception of the dansyl-phenol derivative of Mesurol whose emission maxima shifted from 525 nm to 507 nm after five days, without a decrease in intensity.

Effect of repeated scanning

In earlier work[7,9] it was shown that UV light tends to break down the dansyl derivatives, and hence for best results the reaction and chromatography should be done in the dark. The effect of scanning on the fluorescence intensity of the derivatives has been examined (Fig. 3). Scanning at seven intervals over a five-day period resulted in an initial increase in fluorescence, due to the transfer of the dansyl derivative from the adsorbed state to the triethanolamine as mentioned before. This is followed by a decrease due to the degradation effect of the UV light[7–9]. The results of ten successive scannings within 5 min show a continual decrease with each additional scan, due to the degradation effect of the UV light. Most dansyl derivatives of the carbamates exhibited this phenomenon, with the exception of

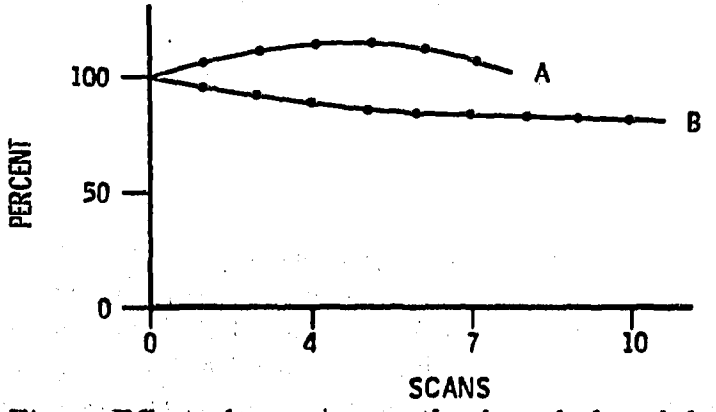

Fig. 3. Effect of scanning on the dansyl-phenol derivative of Mesurol. Curve A = 7 scans over a 5-day period; curve B = 10 immediate scans within a 5-min period.

Zectran and Matacil where a significant decrease was observed with both types of scanning. However, the quantitative potential of the method is not affected, since unknowns and standards are run on the same plate and are usually scanned simultaneously at the same number of times.

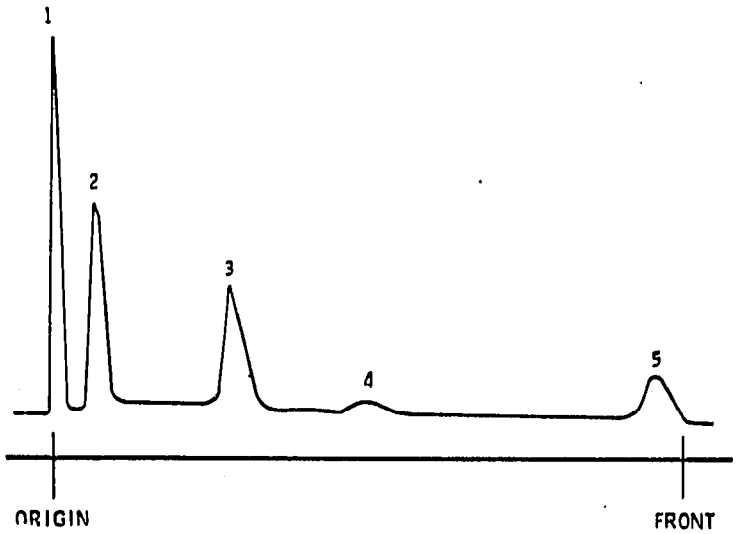

Fig. 4. An actual chromatogram scan for the determination of Mesurol from local lake water. The fluorescence was measured at 525 nm, using the Zeiss 365-nm filter. Peak 1 = dansyl-OH and impurity; peak 2 = dansyl-methylamine; peak 3 = dansyl-phenol; peak 4 = excess dansyl chloride; peak 5 = impurity at the solvent front. Benzene–acetone (98:2) was used as the eluting solvent, on Silica Gel G plates.

Water sample analysis

The water sample analyses were carried out with essentially no interference from co-extractives. In the organic extracts of the samples some gummy residual material was encountered. However, when the reaction is completed and an aliquot is spotted on the plate, the co-extractives remain at the origin. A scan of a TLC from lake water containing Mesurol is shown in Fig. 4. Quantitative results are easily obtained with this system. There was some difficulty in the extraction of the sea water samples due to the formation of a persistent emulsion. Much of this was destroyed, however, by using a glass stirring rod and also by placing a small wad of glass wool up the spout of the separatory funnel which breaks up any remaining emulsion and allows only the methylene chloride phase to pass. Recoveries were not significantly affected by this treatment. The recoveries by this method ranged from 86–106% for each carbamate at the 2-p.p.b. level (Table III). The analysis of the samples containing only Mesurol and the ones containing all three carbamates was carried out on Silica Gel G layers. For analysis of the samples containing Mesurol and Landrin Silica Gel G–silver oxide plates were required[9], since the dansyl-phenol derivatives of these two carbamates are not separable otherwise. In this system the dansyl-phenol derivative of Landrin has an R_F value of 0.55, whereas that of Mesurol has an R_F value of 0.25.

The results indicate that recoveries are sufficiently high at these concentrations to permit analysis of the carbamates. The spiked samples were not allowed to stand for any length of time, since reactions in the water system may destroy the carbamates

TABLE III

RECOVERIES (%) OF CARBAMATE PESTICIDES (2 p.p.b.) FROM WATER SAMPLES

Samples 1 and 2: analysis of the dansyl-phenol derivatives on Silica Gel G, and on Silica Gel G–silveroxide, respectively; sample 3: analysis of the total dansyl-methylamine derivative on Silica Gel G.

Water source	*Sample 1*	*Sample 2*		*Sample 3*
	Mesurol	*Mesurol*	*Landrin*	*Total carbamate*[a]
Tap	93	95	105	95, 104
Lake	106	98	93	86, 94
Sea	98	93	96	90, 96

[a] Landrin, Mesurol and Baygon.

through hydrolysis or oxidation, which would result in misleading recovery data. It must be considered, however, that decomposition of carbamates in natural water samples will occur and usually at a faster rate than for other types of insecticides.

CONCLUSIONS

Dansyl chloride can be a useful reagent for the analysis of N-methyl carbamate insecticides. Natural water samples can be analysed successfully at the 2-p.p.b. level with no interference from co-extractives. Calibration curves were found to be linear up to 300–400 ng per spot of carbamate and extend through the origin. The fluorescence of the derivatives is very stable in the absence of UV light and plates may be stored for long periods before analysis without detrimental effects on the quantitative results. This makes the method suitable as a field method since the reaction and chromatography can be carried out in the field with the plate being analysed quantitatively later.

The sensitivity of this method compares favourably to GC methods but analyses are usually simpler to perform. No other TLC methods for carbamates offer quantitative analysis at such low concentrations. The method also has the advantage of eliminating spray procedures for the actual formation of fluorescent spots and thus background interferences due to irregular spraying are avoided. The actual labelling process can act as a clean-up step in itself for the removal of interferences. For complex samples, two-dimensional chromatography may be used for the separation of the derivatives and quantitative measurements could be made by using an internal standard[11].

The present scope of this work is to determine parent carbamate content and the effect of interferences on the analysis. The method may be adapted to the analysis of certain degradation products. It is also applicable to the analysis of dimethyl carbamates, phenyl carbamates, and substituted ureas, which are currently under investigation.

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