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DETERMINATION OF RESIDUES OF METHOMYL AND OXAMYL AND THEIR OXIMES IN CROPS BY GAS–LIQUID CHROMATOGRAPHY OF OXIME TRIMETHYLSILYL ETHERS

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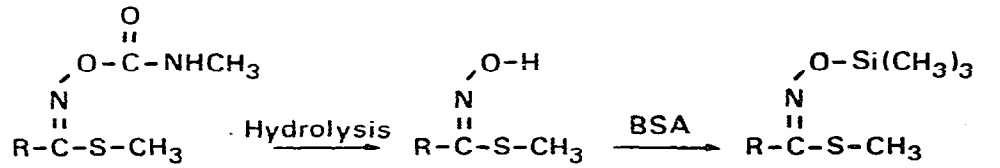
SUMMARY

The gas–liquid chromatographic behavior of methomyl (methyl N-[(methylcarbamoyl)oxy]thioacetimidate), oxamyl (methyl N′,N′-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate), their respective oxime hydrolysis products and the trimethylsilyl (TMS) ethers of the oximes on 5% OV-1 was studied under isothermal conditions using a flame-photometric detector in the sulfur-selective mode. In contrast to the behavior of the parent carbamates and underivatized oximes, the oxime–TMS ethers readily produced symmetrical peaks of consistent size. Quantities of derivative equivalent to at least 0.25 ng of oxime were easily measurable. Derivative formation was reproducible for standards over the range 10.0 to 0.25 μg/ml in benzene and at 10.0 and 0.50 μg/ml in the presence of extractives from tomato, carrot and celery at concentrations equivalent to 10 g/ml of crop. Derivative yields from crop extract fotifications were 89% or better in most cases. Both the carbamates and oximes were simply and consistently recovered in high yield from crops fortified at 1.00 and 0.05 ppm using the procedures described. The inclusion of a second analytical step provided separate analysis for oximes and carbamates. The application of these observations to the analyses of residues in crops is discussed.

INTRODUCTION

Methomyl (methyl N-[(methylcarbamoyl)oxy]thioacetimidate) and oxamyl (methyl N′,N′-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate) are members of a group of insecticidal methyl carbamates which may be considered derivatives of sulfur-containing oximes. The sulfur present has provided a convenient function for detection with sulfur-selective detectors. Methomyl analyses have been carried out indirectly by gas–liquid chromatography (GLC) of the oxime produced by hydrolysis with both microcoulometric and flame-photometric detectors (FPDs)[1–3] and by GLC with electron-capture detection of the 2,4-dinitrophenyl derivative of the methylamine also released on hydrolysis[4]. Direct methomyl analyses by GLC using microcoulometric[5] and flame-photometric detection[6] have been reported. High-pressure liquid

chromatography (HPLC) analyses of methomyl and oxamyl using an ultraviolet absorption detector have been described recently[7]. An indirect analysis of oxamyl by a procedure similar to that described previously for methomyl has been published[8].



carbamate oxime oxime TMS ether

methomyl: $R = CH_3-$ $\overset{O}{\overset{\|}{}}$

oxamyl : $R = (CH_3)_2NC-$

Despite the efforts of these workers, the methods still generally suffer from a lack of sensitivity and/or poor reproducibility at some point(s) in the lengthy extraction and determination steps. Typical minimum quantities of methomyl analyzable by direct GLC were 20 (ref. 5) and 5 ng (ref. 6) and by indirect GLC of the oximes were 25 (ref. 1) and 2.5 ng (ref. 2) which necessitated injections of large amounts of concentrated crop extracts to approach determination of 0.05 ppm or less of residue. The lower limit of detectability of oxamyl oxime by GLC reported to be 0.5 ng (ref. 8) is an improvement over methomyl. The poor reproducibility of some of the methods has been discussed recently by Thean *et al.*[7]. The generally low values and occasional wide range of recoveries they subsequently observed with some crops using a direct analysis by HPLC indicates problems still exist with the extraction procedures.

We wish to report our observations on some of the chemical and chromatographic properties of methomyl, oxamyl, their oxime derivatives and the trimethylsilyl (TMS) ethers of these oximes in the presence of crop extractives and their application to a sensitive method for the reproducible analyses of these materials in crops.

MATERIALS AND METHODS

Instrumentation and analysis

A Tracor MT 220 gas chromatograph fitted with a 120 cm × 4 mm I.D. U-shaped glass column packed with 5% OV-1 on 100–120 mesh Varaport 30 and equipped with an FPD having the usual 394-nm filter for selective sulfur detection was used for analysis. The column was one which had been used generally in our laboratory during the past two years for the analyses of various insecticides in a variety of substrates. It was not treated or conditioned in any special way to do the analysis described here. The first 7 cm of the column were left unpacked to provide a glass liner for the injection port maintained at 200°. Glass wool was not used at the injector end of the column. Nitrogen was used as carrier gas at a flow-rate of 80 ml/min. The FPD was maintained at 200° with the hydrogen and air supplied at 35 and 100 ml/min, respectively, in the now commonly used "inverted mode", *i.e.*, the hydrogen and carrier mixed, to prevent flame out. The detector signal was fed to a strip chart recorder with a sen-

sitivity of 255 mm/mV via an electrometer operating in the range $8–256 \cdot 10^{-10}$ A/mV. The column was maintained at 75 and 130° for the analyses of methomyl oxime–TMS ether and oxamyl oxime–TMS ether, respectively. Various other temperatures and temperature-programmed conditions were used in investigating the chromatographic properties of the oximes and carbamates. Details are given where necessary elsewhere in the text. Retention times are included in Table I. Unknowns were analyzed by the comparison of peak heights with those produced by a suitable concentration range of external standards of derivatized oximes. Those amounts of oxime which represent intact carbamate present (see below) were converted to parent carbamate concentrations by multiplying by 1.54 for methomyl and 1.35 for oxamyl as described previously[1,8].

TABLE I

CHROMATOGRAPHIC BEHAVIOR OF MATERIALS AND REPRODUCIBILITY OF TMS ETHER FORMATION IN BENZENE

ND = not detectable.

Compound	*Reaction time (h)*	*GLC*		*Peak height (mm) for amount injected (ng) (standard deviation)*					
		R_t (min)	*Temp. (°C)*	*20**	*10**	*2***	*1***	*0.5***	*0.25***
Methomyl	—	3.5	140	ND	—	—	—	—	—
Methomyl oxime	—	4.6	75	7	ND	—	—	—	—
Methomyl oxime–TMS ether	16	3.7	75	197 (6.7)	60 (3.6)	108 (1.2)	53 (0.6)	29 (1.5)	17 (2.1)
	16***				70 (2.1)			27 (1.2)	
	430§				66 (3.0)			26 (1.7)	
Oxamyl	—	(5.6)§§	130	ND	—	—	—	—	—
Oxamyl oxime	—	5.6	130	16	6	17	ND	—	—
Oxamyl oxime–TMS ether	16	3.5	130	162 (7.6)	49 (2.5)	109 (2.3)	49 (1.0)	25 (1.5)	14 (2.3)
	16***				47 (1.5)			15 (0.6)	
	430§				42 (3.1)			14 (0.6)	

* Attenuation $128 \cdot 10^{-10}$ A/mV; noise not measurable.
** Attenuation $8 \cdot 10^{-10}$ A/mV; noise 1–2% of full scale.
*** Reacted for 16 h prior to analysis of 430-h sample.
§ Portion of original 16-h reaction stored at room temperature.
§§ Response apparently due to oxime formed by decomposition.

A 60 cm × 4 mm I.D. glass column packed with 2.5% OV-210 on 100–120 Chromasorb W AW DMCS with a carrier gas flow of 80 ml/min was also used in the initial phase of this work to provide a comparison of chromatographic properties. The retention times (temperatures) for the various materials on this column were: methomyl oxime–TMS ether, 0.6 min (75°): methomyl oxime, 1.4 min (75°): methomyl, 3.4 min (130°), oxamyl oxime–TMS ether, 0.8 min (130°) and oxamyl oxime, 1.3 min (130°).

Chemicals and crops

Methomyl, oxamyl, methomyl oxime (methyl N-hydroxythioacetimidate) and oxamyl oxime (methyl N′,N′-dimethyl-N-hydroxy-1-thiooxamimidate) were ≥ 99% analytical standards from DuPont (Wilmington, Del. U.S.A.). Standard solutions of

methomyl oxime were prepared at 100 μg/ml in acetone and benzene. Corresponding standards of oxamyl oxime were prepared in acetone and 10% acetone–benzene. Standards of 154 μg/ml of methomyl and 135 μg/ml of oxamyl which are equivalent to 100 μg/ml of the corresponding oximes were also prepared in these solvents. Suitable dilutions were used for fortification and analysis.

N,O-Bis(trimethylsilyl)acetamide (BSA) was supplied by Chromatographic Specialties (Brockville, Canada). Benzene (ACS reagent, Fisher, Pittsburgh, Pa., U.S.A.), chloroform and acetone (ACS reagent, Caledon Labs., Georgetown, Canada) were distilled in glass in our laboratory, the benzene from potassium permanganate and sodium–lead alloy (dri-Na, Baker reagent) and the latter two from permanganate, only. Persons using the procedures described should be aware that the effects of these solvents on human physiological processes have not been fully examined and appropriate care should be taken to keep exposure to an absolute minimum.

Crop samples were from untreated control plots at our field station.

Derivatization

A 1-ml aliquot of the methomyl oxime or oxamyl oxime standard in benzene or of the crop extract in benzene (obtained as described below) was transferred to a 2-ml screw-cap vial, 10 μl of BSA were added and the vial was closed with a PTFE-lined cap, shaken and allowed to stand at room temperature. Samples were taken directly from the vial for GLC analyses. After preliminary examination of the rate of derivative formation and the stability of the derivatives, samples were allowed to react overnight before analysis.

Triplicate samples of methomyl oxime and oxamyl oxime at concentrations of 10.0, 5.00, 1.00, 0.50 and 0.25 μg/ml were derivatized with BSA. After 16 h the samples were analyzed to determine the reproducibility of derivative formation. The average peak heights and standard deviations are included in Table I. The samples were stored at room temperature and some were re-analyzed after 430 h by comparison with newly derivatized standards to allow for changes in detector sensitivity. The data are tabulated in Table I.

Samples of non-hydrolyzed and hydroxyzed (as described below) crop extractives were fortified with methomyl oxime and oxamyl oxime to yield final concentrations of 10.0 and 0.50 μg/ml in crop extractives concentrations equivalent to 10 g/ml of crop. Aliquots were derivatized with BSA and analyzed. The yield of derivative was determined relative to standards in benzene. The results are summarized in Table II.

Gas chromatographic behavior

The gas chromatographic characteristics of the oximes and their TMS ethers were compared by analysis of suitable amounts of standards using the isothermal conditions described. Typical results are shown in Fig. 1 and are quantitatively summarized in Table I. Injections of the oximes in benzene accompanied by benzene containing BSA (10 μl/ml) were examined for TMS ether formation in the gas chromatograph. Partial, non-reproducible conversion to TMS ethers was observed.

The stability of methomyl and oxamyl to the gas chromatographic process was also examined by injection of suitable standards. Methomyl produced a broad tailing peak similar in shape to that observed previously for the oxime but eluting at 3.5 min

TABLE II

PER CENT YIELD AND REPRODUCIBILITY OF TMS ETHER FORMATION IN THE PRESENCE OF CROP EXTRACTIVES

NA = Not analyzable without cleanup.

Crop extract (10 g/ml)	*Treatment*	*Yield (%) of TMS ether (standard deviation)*			
		Methomyl oxime (μg/ml)		*Oxamyl oxime (μg/ml)*	
		10	*0.5*	*10*	*0.5*
Tomato	NH*	94 (1.7)	102 (2.5)	90 (2.3)	105 (4.9)
	H**	99 (3.2)	97 (1.2)	99 (3.8)	92 (7.4)
Carrot	NH	102 (5.0)	97 (2.7)	79 (6.2)	96 (4.5)
	H	89 (0.6)	96 (1.5)	72 (3.5)	81 (2.7)
Celery	NH	101 (2.1)	109 (4.0)	93 (4.6)	102 (8.7)
	H	76 (4.0)	114 (15.5)	103 (1.0)	—***
Cabbage	NH	100 (5.0)	NA	96 (1.2)	NA
	H	95 (5.0)	NA	NA	NA

* Unhydrolyzed crop extract.
** Hydrolyzed crop extract.
*** Sample not done because of shortage of control crop.

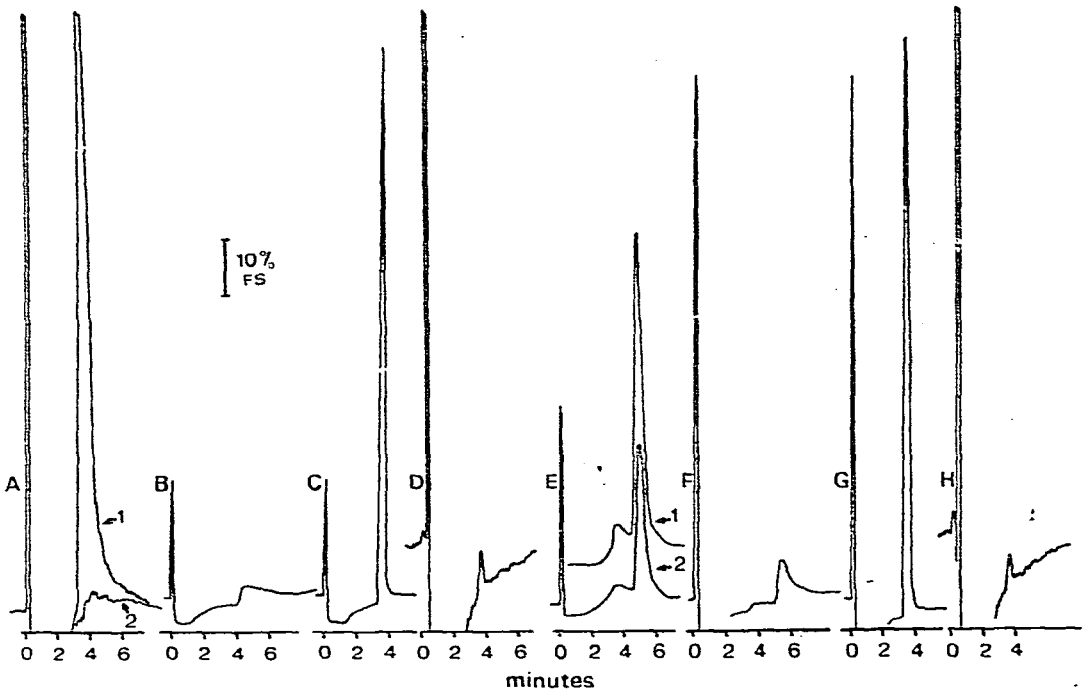


Fig. 1. Gas chromatograms of pure materials and derivatives. A1 = Methomyl, 140°, 30 ng, att. $16 \cdot 10^{-10}$ A/mV; A2 = as A1, 15 ng; B = methomyl oxime, 75°, 20 ng, att. $128 \cdot 10^{-10}$ A/mV; C = methomyl oxime–TMS ether, 75°, equivalent of 20 ng of oxime, att. $128 \cdot 10^{-10}$ A/mV; D = as C, equivalent of 0.25 ng of oxime, att. $16 \cdot 10^{-10}$ A/mV; E1 = oxamyl oxime, 130°, 100 ng, att. $256 \cdot 10^{-10}$ A/mV; E2 = oxamyl, 270 ng (as E1); F = oxamyl oxime, 130°, 20 ng, att. $128 \cdot 10^{-10}$ A/mV; G = oxamyl oxime–TMS ether, 130°, equivalent of 20 ng of oxime, att. $128 \cdot 10^{-10}$ A/mV; H = as G, equivalent of 0.25 ng of oxime, att. $16 \cdot 10^{-10}$ A/mV.

at 140°. Large injections of methomyl (300 ng) were examined using temperature-programmed analysis (75 to 140° at 10°/min after an isothermal hold at the initial temperature for 6 min). Under these conditions 5–10 ng of methomyl oxime would be detectable if present or formed on injection. No response due to oxime was observed for methomyl. Injection of methomyl (300 ng) in benzene after overnight with BSA produced a barely detectable oxime response (about 0.2%).

Oxamyl did not produce a characteristic response when analyzed isothermally at several temperatures between 130 and 175° on OV-1 or when temperature-programmed analyses between these temperatures was used. A response characteristic of oxamyl oxime was observed which indicated 25–50% of the oxamyl injected was converted to oxime in the gas chromatograph under these conditions. Similar behavior was observed on the OV-210 column. Injections of oxamyl (270, 27 or 2.7 ng) in benzene after overnight reaction with BSA produced responses typical of a mixture of oxamyl oxime–TMS ether and oxamyl oxime. The 20–25% conversion of oxime to TMS derivative was similar to that observed for injection of oxamyl or oxamyl oxime accompanied by BSA containing benzene.

Hydrolysis and extraction

Methomyl (154 and 7.7 μg) and oxamyl (135 and 6.8 μg) in 10 ml of acetone were added to a mixture of 20 ml of water and 20 ml of acetone in a 125-ml separatory funnel. After mixing, 3 ml of 0.5 *N* aqueous sodium hydroxide were added which raised the pH to 10–11 as measured with multirange pH paper. After standing at room temperature 1 h, the mixture was acidified with 4 ml of 0.5 *N* hydrochloric acid which lowered the pH to 2–4. The mixture was extracted with 25 ml of chloroform which was dried on separation by passing through anhydrous sodium sulfate. The aqueous acetone mixture was saturated with sodium chloride and extracted with 2 × 25 ml of chloroform which was separated and dried as before. Saturation with salt before the first extraction made subsequent separation of the chloroform–acetone and acetone–brine phases difficult because of the similarity in specific gravity. The drying agent was rinsed with about 10 ml of chloroform, the entire chloroform extract was evaporated to about 3 ml on a rotary evaporator and was solvent exchanged with 3 × 15 ml of benzene. The extract was finally made up to 10.0 ml in benzene. Aliquots were derivatized with BSA and analyzed. Recoveries of the oximes from methomyl and oxamyl were quantitative and 94–96% theoretical, respectively. Methomyl oxime and oxamyl oxime put through the procedure were 94–106% recovered. Standing for 24 h at room temperature at the alkaline stage did not affect the recovery. Recoveries of oximes from carbamates were lower for hydrolysis times less than 1 h at these concentrations of acetone, water and alkali.

Liquid–solid chromatography

A 2.2-cm I.D. glass column packed (bottom to top) with 2 g of anhydrous sodium sulfate and 10 g of aluminum oxide (reagent powder, Matheson, Coleman & Bell, East Rutherford, N.J., U.S.A.) were used. Oxamyl oxime and oxamyl (100 μg) were added to individual columns which were eluted with 100 ml of acetone. Direct analyses of the oxamyl eluted (*i.e.* as its GC decomposition product, oxime) indicated the recovery was 90% or greater. No oxamyl oxime was detectable in the eluate from the other column after BSA derivatization. Triplicate 6.8 μg samples of oxamyl were

similarly chromatographed and the eluates analyzed after hydrolysis, extraction and BSA derivatization. Average recoveries were only 55%. No additional material could be removed from the column with acetone. The chromatography was repeated using aluminum oxide deactivated with 5 and 10% water with similar results.

To evaluate the separation and recovery of oxamyl and oxamyl oxime on aluminum oxide in the presence of extractives, the acetone water extracts of the four crops fortified at 1 ppm were extracted with chloroform without alkali hydrolysis. The chloroform extracts were solvent exchanged to acetone and chromatographed as above. The eluates were reduced to 10 ml, put through the hydrolysis–extraction–derivatization procedures and analyzed. In general the recovery of oxamyl from the column was greatly improved in the presence of the crop extractives. The recovery values are included in Table III.

TABLE III

RECOVERIES OF OXIMES AND CARBAMATES FROM BLENDER AND EXTRACT FORTIFICATIONS

ND = not detectable, NA = not accurately analyzable because of interference at crop concentration of 4 g/ml.

Compound	*Fortification level (ppm)*	*Fortification location*	*Pre-hydrolysis chromatography*	*Recovery (%) (standard deviation)*			
				Tomato	*Carrot*	*Celery*	*Cabbage*
Methomyl	1.00	Blender	No	89 (7.0)	93 (5.7)	97 (8.5)	91 (8.7)
		Extract	No	94	99	—*	110
	0.05	Blender	No	81 (10.4)	89 (3.6)	99 (4.0)	92 (2.9)
Methomyl oxime	1.00	Blender	No	96 (1.7)	102 (0.6)	93 (12.1)	91 (5.8)
		Extract	No	103	97	105	113
	0.05	Blender	No	96 (7.6)	100 (2.3)	100 (10.7)	84 (5.1)
Oxamyl	1.00	Blender	No	97 (1.7)	99 (3.2)	97 (6.2)	100 (5.1)
		Blender	Yes	97	92	87	100
		Extract	No	101	106	106	—
	0.05	Blender	No	105 (0)	103 (8.1)	96 (3.5)	NA
Oxamyl oxime	1.00	Blender	No	97 (3.6)	91 (2.3)	98 (2.0	98 (2.5)
		Blender	Yes	ND	ND	ND	ND
		Extract	No	98	102	—	—
	0.05	Blender	No	106 (7.9)	101 (18.5)	89 (0.7)	NA

* Samples not done because of shortage of control crops.

The chromatography of standards on Florisil was also evaluated using 100-ml mixtures of increasing proportions of acetone in hexane as eluents. Small amounts of oxime were eluted with hexane–acetone (70:30), but the major portion of both the oxamyl and its oxime eluted in the hexane–acetone (60:40) fraction.

Residue analysis: fortification and recovery

Plant material was reduced to a roughly 1:1 acetone–water extract by mascerating 100 g of cut up sample with 100 ml of acetone in a blender for 1 min at 20,000 rpm and 2 min at 13,000 rpm. The macerate was filtered with suction using a Buchner funnel and Whatman No. 1 paper. The blender jar and aluminum foil used as a secondary lid were rinsed with about 35 ml of acetone which was added to the

fibrous mass in the funnel after most of the initial filtrate had been collected. The rinse was allowed to begin to seep through without suction before the vacuum was again applied. The filtrate volume was adjusted to 200 ml in a graduate cylinder by the addition of acetone or the evaporation of a small amount of solvent in a stream of nitrogen as required. Samples were fortified separately with 154 and 7.7 μg of methomyl, 100 and 5.0 μg of methomyl oxime, 135 and 6.8 μg of oxamyl and 100 and 5.0 μg of oxamyl oxime in 10 and 5 ml of acetone, respectively which were added to the macerate after the high speed blending.

A 10-ml aliquot of the acetone–water extract from fortification at the high level was hydrolyzed and extracted as described above. An 80-ml aliquot of the extract from fortification at the low level was mixed with 20 ml of water in a 250-ml separatory funnel and 18 ml of 0.5 *N* aqueous sodium hydroxide was added. After 1 h for hydrolysis, 20 ml of 0.5 *N* hydrochloric acid was added. The acidic solution was extracted as before with chloroform using 50-ml portions and about 20 g of salt to saturate the aqueous phase after the first extraction.

Hydrolyzed extracts of cabbage and some samples of tomato were extracted with a portion of chloroform prior to acidification to remove interferences. When this procedure was used the amount of alkali was increased 2–3 fold to insure that the weakly acidic oxime was as highly ionized as possible to prevent its extraction by the organic phase. The acetone also extracted in this step, about 20 and 40 ml for the high and low levels, respectively, was replaced before the solution was acidified.

In addition to the crop samples fortified at the maceration stage, corresponding amounts of some control extracts were fortified in the separatory funnel prior to hydrolysis. All derivatized extracts (concentrations 0.5–10.0 g/ml) were analyzed isothermally on OV-1 using the conditions described in the instrumentation section except for cabbage. In this case it was necessary to rapidly elute longer retention time components by raising the temperature to 175° after the oxime–TMS ether had eluted at the isothermal conditions. After 10 min at the high temperature the column was reequilibrated for the next analysis. The recovery data for the blender and extract fortifications are summarized in Table III. Typical chromatograms are shown in Figs. 2 and 3.

RESULTS AND DISCUSSION

Chromatographic behaviour and derivative formation

The successful elution of nanogram quantities of a thermally unstable, polar, alkali-labile molecule such as methomyl from a gas chromatography column is dependent on many factors including the temperature, liquid phase and "conditioning" of the column. A comparison of the observations of Williams[5] and Reeves and Woodham[6] with the statement made by Pease and Kirkland[1] on the chromatography of methomyl clearly demonstrate this. Both methomyl and oxamyl are generally considered too difficult to chromatograph reproducibly at the levels required for residue analysis. Fig. 1A is indicative of one type of problem encountered. The oximes produced by the alkaline hydrolysis of these carbamates are alkali stable, weakly acidic, polar molecules possessing greater thermal stability than the parents. This fact has been used to advantage in the indirect GLC analyses of the two carbamates as their oximes[1–3,8]. However, because of their other properties they too are not ideally

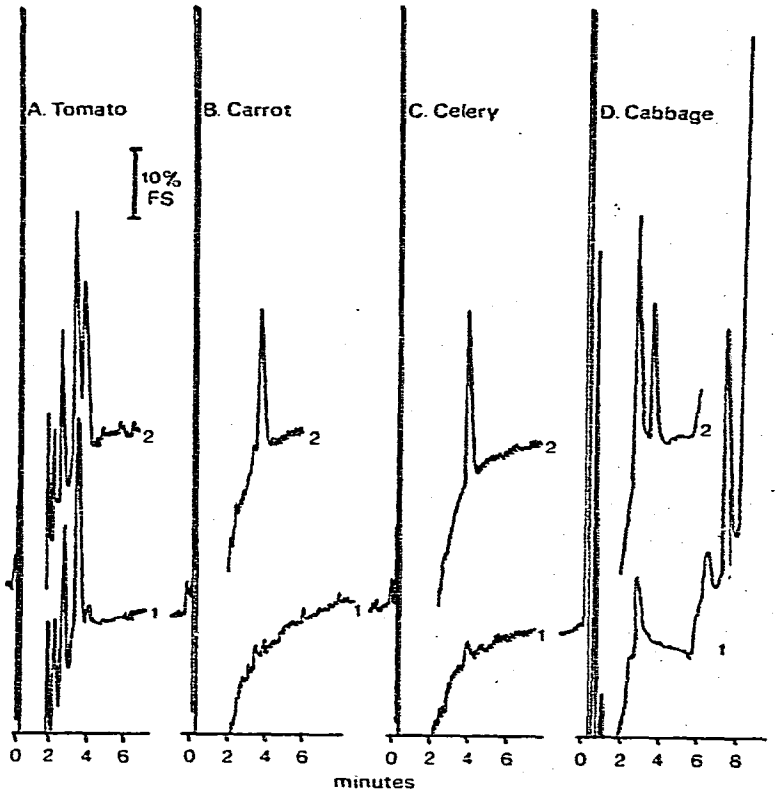


Fig. 2. Gas chromatograms of control and methomyl-fortified crops. A1, B1 and C1 = extract equivalent to 20 mg of control crop, no cleanup; A2, B2 and C2 = same as 1 but fortified with methomyl at 0.05 ppm; D1 = extract equivalent to 20 mg of control crop, chloroform cleanup; D2 = same as 1 but fortified with methomyl at 0.05 ppm.

suited for GLC. Careful column conditioning is required and temperature programming is frequently used to obtain the peak symmetry necessary to provide sensitivity in the low nanogram range. As shown in Fig. 1 the shapes of the responses observed for methomyl and its oxime are not greatly different on the column used in this study and for sufficiently large amounts their heights are also similar. Residue analyses of methomyl and oxamyl are required only periodically at our laboratory and we were interested in removing the requirement for a carefully conditioned and maintained column. The TMS derivatives of polar molecules are easily formed and have been widely used to improve their thermal stability and chromatographic characteristics[9]. We decided to investigate the formation and chromatographic properties of the TMS ethers of methomyl oxime and oxamyl oxime as a first step in improving the analysis. To evaluate the effect of derivative formation on response we chose a column of 5% OV-1 on 100–120 mesh Varaport 30 which had been in use for various residue analyses for about two years. Minimum detectable quantities of methomyl oxime and oxamyl oxime were about 5 and 2 ng, respectively, and the peaks were very asymmetric (Fig. 1, B and F). The TMS ether derivatives formed by the reaction of the oximes with BSA at room temperature in benzene gave sharp symmetrical peaks at shorter retention times than the oximes (Fig. 1, C and G). Minimum detectable

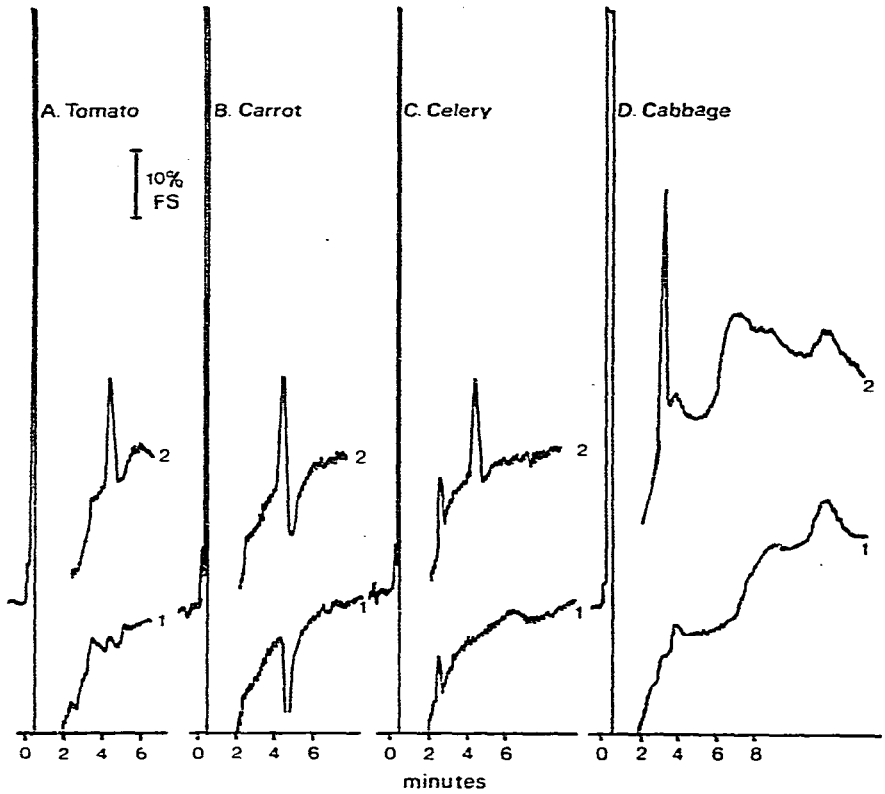


Fig. 3. Gas chromatograms of control and oxamyl-fortified crops. A1, B1 and C1 = Extract equivalent to 20 mg of control crop, no cleanup; A2, B2 and C2 = same as 1 but fortified with oxamyl at 0.05 ppm; D1 = extract equivalent to 2 mg of control crop, chloroform cleanup; D2 = same as 1 but fortified with oxamyl at 1.00 ppm.

quantities were less than 0.25 ng for both compounds (Fig. 1, D and H). It was clear that the derivatives had potential for improving the analysis.

To be useful in residue analysis a derivative must be both easy to prepare reproducibly over a wide range of concentrations in the presence of large amounts of crop extractives and relatively stable. The minimum time required for complete derivatization of 100 μg of oxime in 1 ml of benzene containing 10 μl of BSA at room temperature was difficult to determine by GLC because of the reaction of the oximes with the reagent in the gas chromatograph. Maximum response was not developed in 2 h. We found it convenient to let the samples stand overnight. No further increase in response was observed beyond this time. This time and amount of BSA were sufficient to completely derivatize 100 μg of oxime prior to or on subsequent gas chromatography as no residual oxime could be detected in reaction mixtures analyzed. The reproducibility of derivative formation over the concentration range 10 to 0.25 μg/ml in pure solvent was excellent as is shown in Table I. The peak heights are the average of duplicate injection of triplicate samples. The relatively large standard deviations for the highest concentration are attributed to the well known exponential response of this detector and the resulting sensitivity to minute changes in the amount injected at the 10 μg/ml level. The responses from samples stored at room temperature

for 430 h were not significantly different from newly prepared samples demonstrating the excellent stability of the derivatives in the reaction mixture. A small decrease in sensitivity to oxamyl oxime was observed in the later analyses shown. Such changes were observed with time but were not always decreases. No detrimental effect of the BSA on the sensitivity of the FPD has been observed during the two years we have been periodically involved in this study.

To test the effect of crop extractives on the derivatization reaction, samples of crop (100 g) were reduced to 10 ml of benzene extract by the procedures described but on a large scale, *i.e.* hydrolyzed extracts, and by a slightly modified procedure in which the alkaline hydrolysis was omitted, *i.e.* unhydrolyzed extracts. These extracts were fortified with 5 and 100 μg of the oximes (corresponding to 0.05 and 1.00 ppm on crop) and triplicate aliquots were treated with BSA. The extractives concentration equivalent to 10 g/ml of crop was selected as the maximum interference we would attempt to tolerate. The average yields of derivative are recorded in Table II. In the majority of cases the yields were greater than 90% and showed good reproducibility even at the high concentration of extractives. The cabbage was difficult to impossible to analyze because of interference. These samples had not been cleaned up by the chloroform extraction described because of the purpose of the test. Hydrolyzed celery produced the greatest quantity of extractives and the reproducibility was poor on the low level fortification of both oximes. Some anomalously lower yields were observed, *i.e.* methomyl oxime–1 ppm hydrolyzed celery, oxamyl oxime–1 ppm hydrolyzed, oxamyl oxime–unhydrolyzed carrot and oxamyl oxime–0.05 ppm hydrolyzed carrot, but reproducibility was maintained. This suggests that if required these samples could be analyzed at this concentration by using appropriately fortified controls as standards as we have done for carbofuran analyses where extractives could possibly have a detrimental effect on derivatization efficiency[10]. In subsequent work described below, no difficulty was encountered with the derivatization at extract concentrations equivalent to 4 g/ml. This evidence clearly indicates that the TMS ethers of the oximes are suitable derivatives for residue analyses in crop extracts as long as one is aware of the limitations that may be imposed by the amounts of extractives present.

Extraction and residue analysis

The initially published procedures for the indirect determination of methomyl and oxamyl as their oximes[1,8] reported high average recovery values for numerous substrates. Unfortunately the range of values for some substrates was quite large, *i.e.* 80–115% for methomyl and 73–120% for oxamyl, and no indication was given if the extremes were associated with the extremes of fortification. Ranges of this magnitude limit the credibility of the analytical method. Fung[2,3] modified the procedure slightly and reported improved reproducibility for methomyl. Thean *et al.*[7] recently attacked this problem by direct analyses of the carbamates with HPLC. Using the original procedure to recover the carbamates from fortified extracts they observed considerably lower recoveries, *i.e.*, 79–81% for methomyl and 61–77% for oxamyl. Replicates showed somewhat less variation than the original data but maximum variations of $\pm$ 10–14% were observed in some cases.

We approached this analytical problem with the idea that, if the carbamates could be extracted from crops with a water soluble organic solvent and the hydrolysis to the oximes accomplished in the same solvent, the procedure would be simpler

and perhaps more accurate. The extraction procedure described by Luke *et al.*[11] utilizing acetone for a variety of insecticides suggested its use. Difficulty was anticipated with the recovery of the polar oximes from acetone–water mixtures, but the fact that salt saturation of 50:50 mixtures sufficiently depressed the solubility of acetone in water to cause the separation of two phases indicated recovery might be practical. Chloroform extraction of oxime fortified acetone–water mixtures which were saturated with salt gave reproducible recoveries of 95% or greater. Methomyl and oxamyl were completely hydrolyzed to the oximes after 1 h at room temperature in acetone–water (50:50) mixtures on addition of 3 ml of 0.5 *N* aqueous alkali or in 80:20 mixtures on addition of 18 ml of alkali. Recoveries of the oximes generated from the carbamates were 99–100% and 94–96% of theoretical, respectively. Both oximes and carbamates were recovered in high yield from fortified crop extracts demonstrating that crop extractives did not alter the efficiency of the hydrolysis or extraction. Recovery data for crops fortified at the masceration stage are given in Table III. Typical chromatograms of concentrated extracts (*i.e.*, 4–10 g/ml, injections equivalent to 16–20 mg of crop) are shown in Figs. 2 and 3. In general carrot and celery were problem free. The negative response following oxamyl oxime–TMS ether in carrot varied but was never larger than shown here. The response immediately preceding methomyl oxime–TMS ether in tomatoes varied greatly among samples and in some cases it was necessary to use the chloroform cleanup to reduce it to about the level shown. Methomyl oxime–TMS ether analyses to at least 0.05 ppm could be done on cabbage after chloroform cleanup but 0.05 ppm oxamyl oxime–TMS ether could not be analyzed accurately because of interference. Analyses of more dilute extracts, *i.e.*, 0.5 g/ml, for 1-ppm fortified crops were relatively simple.

Differentiation of carbamates and oximes

The indirect analyses of methomyl and oxamyl as their oximes will be subject to error if the substrate contains the oximes, as well as the carbamates. None of the published procedures provides for a separate analysis of these two components. It has been demonstrated that non-conjugated oximes make up a very small percentage of the total methomyl and oxamyl residues in tobacco leaves[12,13], but the evidence for the absence of methomyl oxime from the treated corn and cabbage foliage is inconclusive[12]. The uncharacterized material from these crops that could be the oxime represents about 1.5% of the dose applied while the amounts of methomyl remaining represent only 6–7%. The inclusion of this 15–25% oxime in a methomyl analysis can alter the final result by as much as 39% when the correction of 1.54 is made to convert total oxime observed to methomyl. The more recent data on oxamyl metabolism in alfalfa, potatoes, apples, oranges and tomatoes[13] clearly shows that its oxime is present in significant amounts compared to the parent carbamate. Reports on the decomposition of methomyl in soil[14] and oxamyl in soil and water[15] have also demonstrated that comparable amounts of parent carbamate and oxime can be present. The procedures described permit a separate determination of the carbamates and their oximes. In the case of methomyl this is easily achieved by a separate analysis of a sample of unhydrolyzed extract for methomyl oximes as its TMS ether. Any methomyl present is not converted to the oxime as its derivative and therefore will not interfere. The amount of actual methomyl in the sample can be calculated from the amount of additional oxime observed in the hydrolyzed sample. For oxamyl it is

necessary to separate the oxime from the carbamate before hydrolysis. Analysis of an unhydrolyzed sample is not specific for oxamyl oxime because oxamyl, if present, is partially converted to the oxime within the gas chromatograph and this oxime is partially converted to the TMS ether producing erroneously high results. Oxamyl was readily separated from the weakly acidic oxime by chromatography on aluminum oxide. The recovery at the 5-μg level for pure oxamyl standards was only 55%, but in the presence of crop extractives recoveries were 90–100% at this level (see Table III). Lower levels were not examined. The 10 g of adsorbent used was sufficient to remove 100 μg of oxamyl oxime from applied standards and the 5 μg present in oxime fortified crop samples. Deactivation of the aluminum oxide with 5 and 10% water did not improve the recovery for applied standards. Extension of the reported separation of aldicarb from its oxime on Florisil[16] to oxamyl was not successful.

CONCLUSION

The TMS ether derivatives of methomyl oxime and oxamyl oxime have been found to be suitable for the determination of the oximes, either naturally present or derived from the carbamates by hydrolysis, in crop extracts by gas chromatography. Their improved chromatographic properties provide at least a ten fold better sensitivity than the oximes under isothermal conditions on the routinely used GLC column examined. The extraction–hydrolysis procedure developed requires much less glassware, solvent, physical manipulation of solutions and time than previously published procedures. Recoveries of 1 and 0.05 ppm of carbamates and oximes from four crops were in the range 89–106% for 28 of 30 samples studied. Replicates showed much less variability than previously reported. The procedures developed permit the analyses of crops for total oxime and carbamate as oxime derivatives for quick survey work, or by the inclusion of a second analytical step, the determination of one component and the calculation of the other by difference.

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